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SECTION 18

LICHENOLOGIE

1. — *PHYSIOLOGIE ET SYMBIOSE DES LICHENS*

RAPPORT SUR LA PHYSIOLOGIE ET LA SYMBIOSE DES LICHENS

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En dehors des traités d'A. L. SMITH (1921), de TOBLER (1925, 1934), de MOREAU (1927), et du récent *Traité de Lichénologie* de H. DES ABBAYES (1951), les lichénologues disposent maintenant de travaux ou d'articles d'ordre général, ainsi que de bibliographies méthodiques et de revues des travaux lichénologiques fort instructives, parmi lesquelles il y a lieu de mentionner notamment les dernières en date, que nous devons à MATTICK et à H. DES ABBAYES.

Aussi bien, sans revenir sur des mises au point excellentes, nous bornerons-nous à évoquer, dans les limites de ce rapport, certains seulement des plus importants problèmes de la physiologie des Lichens.

La physiologie du champignon lichénique. — Avant l'introduction des techniques de culture pure, les champignons lichéniques étaient généralement considérés, par les partisans de la nature double des Lichens, comme des parasites stricts et plus ou moins sévères, incapables de vivre sans leurs gonidies : on avait constaté, en effet, lors des expériences d'analyse ou de synthèse, que la spore germe sans trop de peine, mais que les hyphes qui en sont issues ne peuvent poursuivre seules leur développement, et que, sans le secours de la gonidie, elles ne pouvaient résister à la décomposition, même sur des milieux nutritifs et placées à l'abri d'un excès d'humidité. Les problèmes relatifs à la physiologie de ces champignons apparaissaient alors impossibles à traiter autrement que par voie indirecte ou purement spéculative.

Au contraire, en prouvant qu'ils étaient parfaitement capables de vivre en saprophytes à l'état isolé, la méthode des cultures pures a permis d'aborder directement ces problèmes et, grâce à l'emploi de milieux variés, d'abord non synthétiques, puis partiellement ou totalement synthétiques, de parvenir à des résultats qu'il est intéressant de rappeler brièvement.

Tout d'abord, au point de vue morphologique, les champignons liché-

niques des lichens hétéromères présentent tous, au début, un même mode de développement (hyphes aériennes, conidies, stolons); sur milieu solide, leur croissance aboutit à la formation de thalles dont la morphologie et la structure générales sont celles des lichens correspondants (WERNER). Il en résulte que, abstraction faite de la morphologie fine du thalle, leur potentiel morphogénétique thallin est inscrit dans le patrimoine héréditaire de leur haplophase, qu'il représente une adaptation à la vie symbiotique, et qu'il peut se manifester indépendamment de la collaboration actuelle de la gonidie.

Autant que nous le sachions, car nous ne possédons encore qu'un très petit nombre de résultats obtenus sur des milieux entièrement synthétiques, les champignons lichéniques n'ont pas subi une évolution physiologique régressive, une diminution de leur pouvoir de synthèse ou, si l'on veut, de leur équipement enzymatique, ni bien différente, ni bien accentuée par rapport à celle qu'on connaît chez les champignons non lichénisés, parasites ou même saprophytes: ils réduisent les nitrates, les sulfates, utilisent des sources variées d'azote ou de carbone; comme beaucoup d'autres champignons filamenteux, ils peuvent ou non se comporter en métatrophes pour l'aneurine et la β -alanine, et peut-être pour d'autres facteurs dont le besoin est connu, par exemple chez les Hystériales et les Sphaeriales (biotine, mésoinositol, acide pantothénique); comme ailleurs, on a constaté chez eux des comportements divers vis-à-vis des milieux nutritifs organiques ou minéraux (THOMAS, QUISPEL, ZEHNDER, ENDE).

Il ne semble donc pas, malgré la nécessité de nombreuses recherches nouvelles portant sur les aliments, les enzymes constitutifs et adaptatifs, les facteurs de croissance, les stimulants d'autres champignons lichéniques, et aussi des champignons lichénoïdes réviviscents, des champignons lichénicoles parasymbiotes ou non, des lichens facultatifs et des lichens imparfaits (1), que la lichénisation des champignons ait toujours abouti à la perte de telle ou telle partie bien déterminée de leur pouvoir de synthèse, ni qu'en conséquence elle puisse être définie ou caractérisée sur la base d'une évolution physiologique régressive univoque, du moins au point de vue qualitatif.

On notera, à ce sujet, que les « acides lichéniques », qu'on croyait à ce point propres au métabolisme des champignons lichénisés, que ZOPF les nommait des « Flechtenstoffe », n'apparaissent plus aussi caractéristi-

(1) Nous ignorons entre autres choses si les champignons lichéniques pour lesquels l'aneurine est un facteur indispensable, demandent la molécule entière de cette vitamine ou la pyrimidine et le thiazol, ou seulement un de ces deux corps, ou même encore les substances permettant de les synthétiser.

Il est d'autre part certain que les champignons lichéniques ont conservé des pouvoirs cellulolytiques et pectolytiques (attaque des membranes des gonidies par les haustoria, attaque des gelées par les hyphes chez les Phycolichens).

ques à la lumière des recherches récentes, et que d'ailleurs il est beaucoup de lichens qui n'en produisent pas, les Phycolichens notamment.

Cependant, outre leurs propriétés générales de réviviscence et l'unité que confère à la plupart d'entre eux l'adaptation morphologique à la symbiose, les champignons lichéniques possèdent tous ce caractère commun d'une réduction marquée de l'intensité de leur métabolisme général, réduction qui se traduit par une vitesse de croissance d'autant plus faible qu'ils sont plus profondément lichénisés (2), et ils sont également tous caractérisés par le fait qu'en dépit de la réviviscence et de leurs exigences nutritives en somme limitées, ils ne peuvent certainement pas, à l'état saprophytique, soutenir la concurrence vitale dans les conditions naturelles même les plus favorables, exception faite bien entendu de certains « lichens facultatifs ».

Pour vivre dans la nature, ils ont besoin du « concours précieux » de leur gonidie (WERNER).

Ce serait par conséquent dans un affaiblissement de leurs fonctions vitales essentielles, dans une sorte de sénilité ou de « gérontisme », dont l'amoindrissement de l'agressivité des haustoria lorsqu'on passe des lichens primitifs aux lichens supérieurs fournirait une image, qu'il faudrait chercher le trait physiologique le plus général de la lichénisation des phylums de champignons qui se sont engagés dans la symbiose.

A cet égard et sans préjuger du résultat des recherches futures, une attention spéciale doit être accordée, croyons-nous, à cette constatation que, chez tous les lichens à gonidie bleue ou à gonidie verte étudiés jusqu'ici, qu'il y ait contact direct ou non des cosymbiotes, partout et d'une manière constante, le champignon lichénique emprunte sa métachromatine à sa gonidie, si bien que celle-ci perd en presque totalité, non seulement la métachromatine de son corps central (Cyanophycées) ou de son vacuome condensé (Chlorophycées), mais aussi tout ou partie de la basophilie de son cytoplasme, tandis qu'en revanche on voit les cellules du champignon devenir fortement basophiles au niveau de leur cytoplasme et se charger d'un excès de métachromatine vacuolaire.

Or cette métachromatine constitue certainement une substance de première importance pour la vie cellulaire, que sa partie avide de colorants basiques soit formée d'acides ribonucléiques (ARN), comme nous l'avons constaté chez les Cyanophycées par l'emploi de la ribonucléase, soit que, dans certains cas (vacuoles des champignons), elle puisse être constituée par de l'acide adényltriphosphorique (ATP), comme l'ont soutenu certains auteurs.

Qu'il nous suffise de rappeler ici, tout en renvoyant aux publications

(2) On pense que la vitesse de croissance du champignon lichénique en symbiose est inférieure à sa vitesse de croissance à l'état saprophytique. Cependant, dans ce déficit de croissance, la part n'est nullement faite aux intermittences d'activité des lichens et à la pauvreté des milieux naturels.

spécialisées : 1° Que l'acide ribonucléique ou mieux ses polymères entrent dans la constitution des microsomes, lesquels interviendraient dans la synthèse des protides cytoplasmiques, seraient capables d'autoduplication et pourraient passer de cellule à cellule ; 2° Qu'ils participent à la constitution de mitochondries, où se dérouleraient les oxydations cellulaires et les phosphorylations qui leur sont couplées, ainsi que la genèse de l'A.T.P., accumulateur d'énergie par ses liaisons phosphorées ; 3° Qu'ils interviendraient dans la formation de l'acide adénylique et des coenzymes I et II.

Les gonidies. — Considérées comme essentiellement autotrophes par les tenants des vues de SCHWENDENER, la plupart des gonidies se sont révélées, dès les premières expériences de culture, comme des saprophytes préférentielles, au même titre que de nombreuses algues libres.

D'après CHODAT (1913), les *Cystococcus* ou *Trebouxia*, qui comptent parmi les gonidies les plus répandues, sont capables de prendre leur azote à des composés organiques, principalement à des corps voisins des peptides, pendant que le carbone doit leur être fourni, dans la mesure du possible, par une source glucidique, le glucose de préférence, les acides organiques s'étant montrés des aliments carbonés médiocres. La lumière favorise le développement saprophytique, sans qu'on puisse invoquer l'appoint de la photosynthèse, car celle-ci serait abolie en présence de glucose.

Très généralement, les nitrates ne sont pas réduits en présence de glucose, même à la lumière, ou ne donnent qu'un développement insignifiant, inférieur à celui qu'on peut obtenir avec des sels ammoniacaux.

Sur les milieux inorganiques sans addition de glucides, ces gonidies demeurent à peu près stationnaires, et paraissent incapables d'assimilation chlorophyllienne, si bien que CHODAT, se référant aux résultats de JUMELLE (rapport O_2/CO_2 souvent supérieur à 1 chez les Lichens), se demandait si les champignons lichéniques n'exciteraient pas leur cosymbiote à un excès de photosynthèse.

Il a été prouvé également par divers auteurs que le comportement des gonidies ne relève pas d'un type uniforme : les *Coccobotrys* ne peuvent vivre en saprophytes, les *Coccomyxa* préfèrent une nourriture inorganique, les *Stichococcus*-gonidies semblent moins saprophytes que les *Stichococcus* libres, certaines gonidies préfèrent les sels d'ammonium aux peptides ou aux acides aminés, d'autres acceptent l'acétamide, et chez les *Cystococcus* eux-mêmes, où se manifestent des préférences pour des milieux de culture non synthétiques variés, il est des clones (*Cystococcus* de *Caloplaca*) qui réduisent les nitrates en présence de glucose.

A ces connaissances, les recherches récentes effectuées sur des milieux synthétiques ont apporté d'importants compléments. Il a été notamment mis en évidence, sur deux gonidies, que celles-ci, qui paraissent ne pas avoir besoin de facteurs de croissance mais seulement de stimulants,

peuvent être induites à se développer autotrophiquement, sans autre aliment carboné que l'anhydride carbonique, par l'addition à leur milieu de culture de quantités suffisantes d'acide ascorbique ou de corps voisins à fonction diénolique, et cela d'autant mieux qu'on élimine l'oxygène de l'atmosphère de la culture, qu'on y introduit une proportion élevée de CO_2 , et qu'on y crée une forte pression partielle d'hydrogène (QUISPEL).

Ce résultat, qu'il faudrait étendre à de plus nombreuses gonidies, demande certainement aussi une confirmation par l'étude des échanges gazeux de la gonidie en culture, ainsi qu'une interprétation plus poussée. S'agit-il d'une action spécifique des composés diénoliques ou simplement d'un abaissement du potentiel d'oxydo-réduction, comme semble l'indiquer l'action favorisante de l'hydrogène en présence d'un corps électro-actif ? L'action favorisante s'exerce-t-elle sur l'une des phases, lumineuse ou sombre, de la photosynthèse ou sur les deux ? Porte-t-elle sur la réduction du nitrate de la solution de Beijerinck ? Autant de questions.

Quoi qu'il en soit, une fois de plus les gonidies se révèlent semblables aux algues libres, car ONDRATSCHEK avait précédemment découvert l'action de l'acide ascorbique sur ces dernières, tandis que s'ouvre d'autre part une très intéressante voie vers l'explication de l'activité remarquable de la photosynthèse chez les lichens.

Nous ajouterons que les recherches expérimentales ont confirmé que les gonidies sont réviscentes comme les champignons lichéniques, que le maximum de leur activité se situe un peu en deçà de la saturation en eau comme on le savait déjà par les recherches sur les échanges gazeux des lichens, que leurs exigences à l'état saprophytique sans être identiques à celles de leurs cosymbiotes n'en sont cependant pas complémentaires, mais que de toute manière elles se distinguent de ces derniers par leur capacité de vivre à l'état libre.

La spécificité des gonidies. — Evidente quand on s'arrête aux ordres, aux familles, souvent aux genres et parfois aux espèces des gonidies *in situ* (encore que les descriptions de celles-ci, comme leur culture par des moyens simples, aient été ordinairement négligée), la question de la spécificité des gonidies se complique singulièrement si l'on tient compte des très nombreuses races ou clones que les cultures pures ont mises en évidence. En présence du nombre très élevé des espèces de champignons lichéniques par rapport au petit nombre de gonidies morphologiquement discernables, on peut en effet se demander quel choix plus ou moins strict les champignons effectuent parmi les races ou les clones de ces dernières ou, en d'autres termes, quelle est l'importance au point de vue de la symbiose lichénique de ces races ou de ces clones.

Diverses objections ont été faites récemment à la notion d'une spécificité stricte ou assez stricte des gonidies telle qu'elle ressortait des travaux de CHODAT, de JAAG et de WARREN : on peut penser toutefois que

les auteurs se sont précisément adressés à des genres de champignons lichéniques (*Chaenotheca*, *Cladonia*) chez lesquels la spécificité gonidiale est assez lâche, et qu'il y aurait sans doute lieu d'introduire en lichénologie la notion d'hôte normal, bien connue des parasitologues. Comme le montrent surabondamment les parasymbiotes, il n'est nullement exclu au demeurant que divers champignons de nature lichénique puissent être adaptés à une seule et même gonidie.

Au surplus, nous mentionnerons qu'il nous a été possible de trouver de nombreuses races de *Nostoc* et de *Gloeocapsa* distinctes par le pouvoir d'absorption de leurs gelées pour l'iode (*Nostoc commune*, par exemple, se présente sous deux et même trois races si l'on tient compte de la var. *flagelliforme*), et d'établir que les gonidies des espèces et des variétés bien définies de Collémacées et de Pyrénopsidacées se comportent d'une manière constante vis-à-vis de l'iode.

Certes le problème de la spécificité des gonidies appelle de nouvelles recherches, qui seront abordées avec plus de fruit lorsqu'on saura régulièrement réaliser les synthèses de lichens. D'ores et déjà cependant on peut dire qu'il est fondamental, aussi bien pour la notion même de lichen, que pour la solution du problème physiologique de la symbiose, puisque celle-ci se présente histologiquement comme un parasitisme atténué du champignon et pose en conséquence le problème de la spécificité parasitaire, et que d'autre part la nature de la gonidie intéresse profondément le patrimoine héréditaire de son cosymbiote, ce qu'on peut inférer du fait que seules certaines familles, d'ailleurs élevées en organisation (Peltigéracées, Pannariacées, Stictacées), admettent à la fois des gonidies vertes et des gonidies bleues.

La synthèse des lichens. — Depuis que la synthèse naturelle des lichens a été soigneusement observée et décrite (WERNER), les expériences de synthèse ne peuvent plus viser exclusivement une démonstration en quelque sorte superfétatoire de la nature double des lichens; elles doivent tendre à nous munir d'une technique régulière d'obtention de thalles d'origine et de composition bien déterminées, afin de rendre possible l'attaque expérimentale de divers problèmes physiologiques, notamment la question de la spécificité des gonidies, et surtout celle de la nutrition du thalle entier, au sujet de laquelle nous n'avons guère jusqu'ici que des vues restreintes ou purement spéculatives.

Les récentes expériences de synthèse, dont certaines ont été couronnées de succès, nous ont fourni quelques renseignements précis: 1° il apparaît évident qu'on ne peut réaliser une synthèse dans un milieu saturé d'eau, c'est-à-dire dans des conditions dont on sait depuis longtemps qu'elles sont incompatibles avec la stabilité du complexe lichénique; 2° il apparaît également que les conditions artificielles de la vie saprophytique

ne sauraient convenir, qu'il s'agisse d'une trop grande richesse du milieu ou de sa composition.

D'après certaines de nos observations sur des lichens marins des côtes méditerranéennes, nous dirons qu'il ne nous semble nullement nécessaire de réaliser au laboratoire le rythme imbibition-siccité, mais simplement de maintenir une imbibition inférieure à la saturation du thalle. Par ailleurs, nous pensons que les milieux naturels dans lesquels vivent les lichens pendant leurs phases d'hydrobiose (par exemple les solutions de terre pour les lichens terricoles) doivent être pris pour modèles de départ en vue de confectionner des milieux partiellement ou totalement synthétiques.

Théories de la symbiose. — Depuis SCHWENDENER, on a tenté à maintes reprises, selon les connaissances du moment, de rendre compte de la possibilité de la symbiose lichénique par une complémentarité des besoins des cosymbiotes, et accessoirement par une protection que le champignon lichénique assurerait à la gonidie, au moins dans les conditions stationnelles les plus dures.

Ces tentatives, qui toutes manquaient de bases suffisamment étendues, ont abouti finalement à une impasse : les exigences des cosymbiotes se sont révélées diverses selon les cas, et, dans un même lichen, non pas complémentaires, mais à peu près identiques, aussi bien au point de vue de la nutrition qu'au point de vue écologique.

La notion même d'échange de glucides en faveur du champignon a été compromise, du moins pour les lichens à gonidies-*Cystococcus*, jusqu'au moment où QUISPEL a pu montrer qu'une chute du redox du milieu pouvait rétablir la nutrition purement minérale de ces gonidies.

On attire maintenant l'attention sur l'échange de vitamines. Cependant, remarquons-le, cet échange, même s'il était général, expliquerait bien à lui seul un certain satellitisme entre les cosymbiotes, mais sans pouvoir rendre raison totalement de la véritable symbiose lichénique.

Nous faisons appel nous-même à l'intervention des acides nucléiques ou de leurs dérivés en provenance de la gonidie et nous insistons sur le caractère constant du phénomène.

Par ailleurs, on a fortement réduit l'importance des arguments relatifs à la protection que les cosymbiotes s'accorderaient entre eux, grâce aux membranes fongiques (thalles exogènes) ou aux membranes algales (thalles endogènes) : à notre avis, le rôle de ces membranes est d'un autre ordre (adsorption et concentration des solutions nutritives naturelles, défense du chondriome contre la cavulation provoquée par l'hypotonicité du milieu, éventuellement protection de la chlorophylle contre les rayons nocifs du spectre solaire, etc.) et ne représente qu'une des multiples conditions secondaires de la symbiose lichénique.

En fait, comme l'ont largement confirmé L. GEITLER et TSCHERMAK, le champignon lichénique est toujours un parasite plus ou moins atténué de

la gonidie. A la limite, chez les lichens supérieurs, où se manifeste la plus haute adaptation de l'haplophase à la vie symbiotique, ses attaques se réduisent à une simple mise en contact temporaire des cytoplasmes des cosymbiotes, dont les relations deviennent alors morphologiquement semblables à celles des cellules d'un être génétiquement homogène.

L'intelligibilité de la symbiose lichénique relève donc en premier lieu de celle du parasitisme en général, que l'évolution physiologique régressive des parasites n'éclaire encore que très partiellement; elle relève aussi d'une explication encore inconnue de l'atténuation ou des fluctuations de la pathogénicité du parasite vis-à-vis de son hôte, sur lesquelles nous ne savons encore que fort peu de choses (nous ignorons tout des antigènes et des anticorps des cosymbiotes et nous n'avons que des connaissances très restreintes sur leurs systèmes enzymatiques et sur leurs antibiotiques respectifs).

De surcroît, les particularités de cette symbiose, qui s'est établie entre des êtres réviviscents, dont l'un, le champignon lichénique, se trouve dans un état particulier de ralentissement ou de sénilité de son assimilation et de toutes ses fonctions générales, réclament encore une interprétation positive.

Enfin, une théorie complète devra dégager le ou les facteurs limitatifs qui règlent l'équilibre physiologique et l'unité des formes supérieures de lichens.

On le voit: nombreux et difficiles sont encore les problèmes qui s'offrent à la sagacité des chercheurs.

Ignoramus, sed non semper ignorabimus!

3. — REFORME DE LA TAXINOMIE DES LICHENS

THE GENERAL TAXONOMY OF LICHENIZED FUNGI

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RAPPORT

All lichenologists are certainly aware of the fact that very large parts of the different lichen systems now in use are only provisional. Even after a superficial study one finds that the arrangement of families, genera, etc., is often based on untenable or very debatable principles. There are many very fundamental problems which have been treated very little, and extensive and careful investigations are urgently required in many parts of the system.

In a discussion of the general questions about the taxonomy of lichens, or more correctly of the lichenized fungi, we should perhaps at first clarify our attitude to certain basic principles, which have been differently treated in the recent lichenological literature, even if they seem very clear to many lichenologists.

Systems based on the whole lichens, i.e. the lichen-fungi + the lichen-algae are biological systems, not taxonomical ones. This was clearly emphasized by SERNANDER (*Sv. Bot. Tidskr.*, 1, 100, 1907), THOMAS (*Beitr. Kryptogamenfl. Schw.*, IX, 169, 1939), a.o. Lichen systems established by authors regarding the lichens as unitary — not composite — organisms are, of course, very valuable if they are carefully made and based on a profound knowledge of the lichens themselves as well as the lichen literature. We know very well that the lichen-algae often may serve as good indicators for the taxonomical arrangement of the lichen-fungi (cf. JAAG in *Ber. Schw. Bot. Ges.*, XLII, 724-739, 1933). The names given to lichens have been considered as taxonomical names. Therefore it seems to be the most practical procedure for nomenclatural purposes to consider these names as applying to the fungal components.

During recent years some authors, e.g., MATTICK (1950, 1954), and CIFERRI & TOMASELLI (1952, 1953) have contended that we should give names to the lichen-fungi, different from those of the lichen, e.g., *Xanthoriomyces* — *Xanthoria*, *Magnussoniomyces* — *Magnussoniolichen*, *Giacominia* — *Santessoniolichen* subgen. *Para-Santessoniolichen*. The question if we really need these two different classification systems will probably be discussed for a long time. Most lichenologists will certainly prefer the more simple way of adding a small suffix like (lich.), (fung.), or (fung. cult.) after the common name in the very few cases where there is

a chance of misunderstanding what is meant (cf. DU RIETZ 1945). The rejection of all lichen names from the taxonomy would have very far-reaching consequences.

The establishment of higher taxonomical units on exclusively biological characters is undoubtedly untenable. The lichenized fungi must not be separated into different genera distinguished on their different symbiotic algae. Several lichen-fungi, parasymbionts, and parasites (or saprophytes) are referred to different genera on basis of their different mode of nutrition, e.g., *Buellia* — *Karschia*, *Catillaria* — *Scutula*. In recent times have even families been established on such a basis e.g., *Pseudocaliciaceae* (NADVORNIK in *Preslia*, XVIII-XIX, 114, 1939-40). It must be observed, however, that some genera which seem to be such biological parallels are distinguished by fundamental anatomical characters.

It is very natural that in older times the purely vegetative characters of the lichens were very important for the taxonomy. However, our present knowledge of the ascocarps of the lichens shows conclusively that the state of development of the lichen thallus is of a secondary taxonomical importance. Morphologically comparable types of thalli are found in very different natural taxonomical groups, cfr. e.g., *Dirina* — *Roccela*, *Caloplaca* — *Teloschistes*. Nevertheless, we find in recent lichenological literature, even such high taxa as suborders based on exclusively morphological thallus characters, e.g., *Radiati* and *Tabulati* (TOMASELLI, *Arch. Bot.*, XXV, 13, 1949). The importance of the vegetative characters was drastically overestimated by RASANEN (*Acta Bot. Fenn.*, XXXIII, 6, 1943) when he interpreted the resemblance (?) between an elongate stipe of a *Gyrophora* and the thallus of an *Alectoria* as an indication of relationship.

Several facts have been revealed which show clearly that the groups *Pyrenocarpeae* (*Pyrenolichenes*) and *Gymnocarpeae* (*Discolichenes*) are not natural units. On the basis of the different mode of formation of the ascocarps NANNFELDT (1932) established the two groups *Ascoloculares* and *Ascohymeniales*. Within each of these groups there are genera having ascocarps which have been called perithecia and apothecia (cfr. SANTESSON in *Symb. Bot. Ups.*, XII, 44-45, 1952). Recently LUTTRELL (*Univ. Missouri. Stud.*, 24.3.1952) divided the *Euascomycetes* in two groups, *Bitunicatae* and *Unitunicatae*, characterized by the double and simple ascus wall respectively. With a few exceptions these two groups correspond to *Ascoloculares* and *Ascohymeniales*. Very important investigations of the apical apparatus of the asci have been carried out by CHADEFAUD (1942, etc.). A number of lichens were studied from the same point of view by MAGNE (*Rev. Bryol. Lichénol.*, XV, 203-209, 1946). It is very interesting to find that with some exceptions the fungi with ascolocular ascocarps possess asci of « le type à nasse apicale », and those with ascohy-

menial ascocarps have asci of « le type à anneaux apicaux ». A careful study of the parallelism of the mentioned three bases for classification seems to be at present one of our most important tasks.

The anatomy of the ascocarps of the lichens is imperfectly known. We attach a very great taxonomic importance to the presence or absence of algae in the margin of the apothecia. But isn't the difference between the protolecideoid apothecial type on one hand and the supralecideoid and the lecanoroid type on the other hand much more fundamental than the difference between the lecideoid and the lecanoroid types? The excipular tissue of the apothecia should be more carefully studied than is usually made. Dimidiate or entire perithecia are regarded as very important characters. But a study of the origin or nature of a dark basal perithecial wall is usually neglected. Isn't the tissue which is usually called involucrellum of different origin in certain cases? A profound comparative study of the anatomy of the ascocarps would certainly be most valuable for the taxonomy.

From the non-lichenized fungi we know that there are different types of polyspore asci: truly polyspore asci, polyspore asci due to spore division, and due to conidial formation (MARTENS in *Bull. Soc. Myc. France*, LII, 1937-38). We find these types represented among the lichenized fungi as well. Their different taxonomic value should be carefully considered.

For a long time the high taxonomic value of the ascospore types, the spore septation, etc. has been generally admitted. It is perhaps not astonishing that sometimes the importance of certain spore characters has been overestimated. The polarilocular spores for a long time have been regarded correctly as one of the most important characters for the families *Caloplacaceae* and *Teloschistaceae*. There are a number of lichen genera characterized by lensformed or round lumina of the spore cells. This type, which is rare among the non-lichenized fungi, but rather common among the lichenized fungi, seems to be characteristic for certain natural groups, e. g. for the families *Pyrenulaceae* s. str., *Graphidaceae* s. str. (cfr. SANTESSON, *op. cit.*). The occurrence of secondary alterations within a certain spore type should always be observed in order to avoid misinterpretations.

A new way of classification of the lichens was tried by CHOISY (*Bull. Mens. Soc. Linn. Lyon*, 19-22, 1950-53) who established the orders *Acropycnoconidiei* and *Pleuropycnoconidiei* on the types of the conidio-phores in the pycnids. The mentioned work does not contribute to the progress of taxonomy, in spite of the fact that conidial organs are important for the taxonomy and ought to be the object for a renewed and careful study.

In taxonomical works on lichens it is often emphasized that the author has tried to be « consequent » or « logical » in his application of certain characters. However, a character which is of a great importance in one part of the system might well be of less importance in another.

It is very regrettable that probably in no other group of plants is the taxonomic work carried out more carelessly than in lichenology. New arrangements are established often on pure theoretical speculations. Much more than half of the number of new genera established in recent times is due to insufficient knowledge of the lichens and of the literature. Many revelations of the true nature of genera based on mistakes are on the other hand completely neglected. Taxonomy makes no progress by the careless establishment of a lot of new genera, species, etc. Carefully made monographs of entire groups are most urgently required in lichenology.

DISCUSSION SUR UNE CLASSIFICATION NATURELLE
DES ASCOLICHENS

par Maurice CHOISY

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Une telle classification est recherchée par l'auteur depuis près de 40 ans; il est arrivé à considérer comme essentiels les points suivants :

1. IMPORTANCE DE LA PYCNOCONIDIE EN SYSTÉMATIQUE. Cette importance est démontrée par le fait que les pycnoconidies courtes et droites, naissant sur stérigmates articulés, représentent le seul caractère constant dans le phylum des *Theloschistaceae* : le thalle peut être usnéoïde, alectorioïde, membraneux ou crustacé et même subnulé; l'apothécie peut être lécanorine, aspicilioïde ou biatorine, rarement lécidéine; la spore peut être pluriseptée, bi- tri-septée, uniseptée ou simple, à cloisons épaissies ou minces.

C'est ce même caractère pycnoconidial qui relie *Theloschistaceae*, *Physciaceae*, *Parmeliaceae*, *Stictaceae* et *Collemaaceae*; tout genre ou espèce qui ne satisfait pas à cette condition est à exclure de ce groupe naturel.

2. THÉORIE DE L'ÉVOLUTION RÉGRESSIVE. Le phylétisme régressif des Lichens est révélé par la rareté relative des caractères supérieurs : thalles fruticuleux, apothécies composées, spores mûrifformes ou spores simples mais plurinucléées, ou spores très nombreuses. Il est mis en évidence d'une part par la répartition géographique de certaines espèces (*Oropogon loxensis* par exemple), et par la statistique. Exemples :

a) *Oropogon* 1, *Cornicularia* 9, *Alectoria* 32, *Cetraria* 56, *Parmelia* plus de 600 espèces; — b) *Astrotheliaceae* 87, *Trypetheliaceae* 160, *Pyrenulaceae* près de 1.000 espèces; — c) *Polyblastia* 77, *Thelidium* 92, *Verrucaria* 244 espèces.

3. ABSENCE TOTALE DE CRITÈRE AYANT UNE VALEUR ABSOLUE. Présence simultanée des morphologies usnéoïde et alectorioïde chez les *Usneaceae* (sensu Zahlbr.), les *Cladoniaceae* et les *Roccellaceae*. — Absence de limites précises entre les thalles membraneux et crustacés; entre les apothécies des types *Lecanora*, *Aspicilia*, *Lecidea*. Présence d'apothécies arrondies chez les *Graphidineae* et d'apothécies difformes chez les *Cyclocarpineae*; présence simultanée d'apothécies simples et composées chez les *Umbilicariaceae*, les *Pertusariaceae* et les *Thelotremaaceae* (entre autres). Absence de limites précises entre les différents types de spores, qu'il s'agisse du nombre et de la direction des cloisons, ou de l'épaisseur des membranes; analogies cytologiques entre les spores simples binucléées (les plus communes) et les spores uniseptées comme entre les spores du type *Pertusaria* et les spores mûrifformes.

4. POLYPHYLÉTISME CERTAIN ENTRE PYRENOCARPEAE ET CYCLOCARPEAE. Phylum évident reliant les types *Pyrenula*, *Thelotrema*, *Graphis*; enchaînement probable entre *Microglaena* et *Urceolaria* (*Disploschistes*), également entre *Trimmatothele* et *Acarospora*.

5. DISTINCTION DE LICHENS VRAIS, ayant eu une évolution propre comme des végétaux autonomes, et de Pseudo- ou Néolichens affinés à des groupes Ascomycètes non lichénisés; — corollairement, antinomie possible entre genres saxicoles des régions tempérées et genres corticoles ou foliicoles des tropiques, cette distinction étant cependant sujette à interprétation. Il y a vraisemblablement lieu d'éloigner les *Gyalectaceae* saxicoles des genres *Dimerella*, *Semigyalecta*, etc.

6. GONIDIES. Abandon presque total de l'importance systématique de cet organe. Comme conséquence, fusion possible ou au moins rapprochement entre *Acarosporaceae*, *Heppiaceae* et *Pyrenopsidaceae*, ces derniers se distinguant par la pycnoconidie des *Collemaceae* proprement dits.

7. Présence de faux Lichens sous forme d'Algues parasitées par des Ascomycètes. Critère : vie en commun d'Algues pures et d'éléments parasités décrits comme Lichens (*Ephebe*, *Pyrenothrix*, *Coenogoniaceae*).

Selon ces considérations, l'auteur a composé une classification basée sur un polyphylétisme nombreux, dont les principaux groupes sont :

PROTOLICHENES : *Sphaerophorineae*, *Thammodictyaceae*, *Roccellineae*, *Usneineae*.

ARCHAELICHENES : *Peltideales*, *Endocarpoideae* (*Endocarpales*, *Urceolariales*, *Pertusariales*, *Acarosporales*), *Pleuropycnoconidiei* (*Coralloideae*, *Umbilicariales*, *Parmeliales*), *Acropycnoconidiei* (*Cladoniales*, *Rhizocarpales*, *Bacidiales*).

NEOLICHENES : *Thelotremineae* (*Ascohymeniales*); *Opegraphineae* (*Ascoloculares*); *Caliciales*.

PSEUDOLICHENES, ceux-ci se décomposant en *Paralichenes*, *Mycolichenes* (Ascomycètes lichéniformes) et *Phycolichenes* (Algues parasitées).

PROPOSITIONS POUR L'ETABLISSEMENT D'UNE NOMENCLATURE UNIFORME DES ANOMALIES (BILDUNGSABWEICHUNGEN) CHEZ LES LICHENS

par VITUS GRUMMANN

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I. — Les ARGUMENTS, sur lesquels s'appuie ma proposition de caractériser les anomalies ou cas tératologiques (*Bildungsabweichungen*) chez les Lichens par une nomenclature uniforme, sont les suivants :

1°) Les anomalies sont aussi importantes pour la systématique que pour la phylogénie.

2°) Les anomalies n'ont pas été jusqu'ici suffisamment mises en évidence dans les travaux systématiques ou floristiques (au contraire des unités taxonomiques qui ont des noms spéciaux). Elles ont été mentionnées dans le texte concernant chaque espèce, où elles sont plus ou moins cachées. De ce fait, il est difficile de trouver les passages dont on a besoin, concernant les anomalies d'une espèce.

3°) Les règles de la nomenclature (termes taxonomiques) sont inutilisables pour mettre en évidence les anomalies, parce qu'elles ne constituent pas des unités taxonomiques. L'article 77 du code de nomenclature rejette à juste titre les noms qui sont basés sur des monstruosité. Cela concerne donc les anomalies *sensu latiore*.

II. — La RÉALISATION du projet peut être envisagée de la manière suivante :

1°) Le nom de l'anomalie est attaché au nom de l'unité systématique.

2°) La citation de l'anomalie est précédée de l'abréviation d'un terme employé uniformément. Après discussion avec divers collègues, les termes suivants ont été éliminés : *aberratio*, *abnormitas*, *anomalia*, *lusus*, *modificatio*, *monstrositas*, de même phénomène pathologique ou tératologique. Leur signification est ou bien trop précise, ou bien différente par l'emploi qui en a été fait antérieurement.

3°) En conséquence, on propose l'adoption du terme « *tératologie* » dans son sens le plus large, dont on extrait la racine *teras* abrégée en *ter*.

4°) Le nom de l'anomalie suivra le mot *ter*. Pour réduire les noms au minimum, on appliquera toujours le même nom à la même anomalie, se produisant chez toutes les unités systématiques. De la sorte, on ne dépassera pas, pour les Lichens, le nombre de 35 noms.

5°) Parfois un deuxième nom pourra suivre, pour préciser le premier.

6°) On ne choisira pas des noms arbitraires, mais seulement des noms à sens caractéristique de l'anomalie.

7°) La citation d'un nom d'auteur, après le terme désignant une anomalie, pourra être omise, car ces termes n'ont pas de valeur taxonomique.

8°) Les noms des anomalies devront porter le suffixe *-um*, afin de respecter l'accord avec le terme *teras*.

III. — Exemples : 1°) Pour indiquer l'existence d'apothécies secondaires, croissant sur les primaires et développées après la morsure d'animaux : *Lecanora subfuscata* Magn. *ter. secundarium*; *Xanthoria parietina* (L.) Th. Fr. *ter. secundarium rosodisci*; *Diploschistes scruposus* (Schreb.) Norm. var. *bryophilus* (Ehrh.) Müll. Arg. *ter. secundarium receptaculi*. — 2°) Pour le développement de proliférations aux points de rupture du thalle, ou encore aux blessures, prenant leur origine dans la formation des

soralies (à ne pas confondre avec la formation de jeunes thalles à partir des sorédies): *Alectoria jubata* (L.) Ach. var. *prolixa* Ach. ter. *adventitium thalli*; *Alectoria jubata* (L.) Ach. var. *prolixa* Ach. ter. *adventitium soralii*.

IV. — BIBLIOGRAPHIE. Les arguments cités et la possibilité de les mettre en œuvre ont été traités récemment dans mon article: « Zu einer einheitlichen Benennung von Bildungsabweichungen bei den Flechten » (*Ber. Deutsch. Botan. Gesells.*, 1954). J'y ai joint une liste de toutes les anomalies connues chez les Lichens et je leur ai appliqué la terminologie proposée ici, avec l'emploi du mot *teras* abrégé en *ter*.

4. — PHYTOSOCIOLOGIE ET GEOGRAPHIE DES LICHENS

RAPPORT SUR L'ETUDE PHYTOGEOGRAPHIQUE ET PHYTOSOCIOLOGIQUE DES LICHENS

par P. DUVIGNEAUD

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1. REMARQUE PRÉLIMINAIRE.

Le présent rapport s'inspire de ce qui a été écrit par H. des ABBAYES, sur la phytogéographie des Lichens, dans son remarquable traité de Lichénologie. En fait, nous n'avons rien d'important à ajouter aux considérations émises par cet auteur, considérations qui constituent une excellente base de discussion.

2. PHYTOGÉOGRAPHIE.

La phytogéographie des Lichens a été sous-estimée et l'est encore aujourd'hui dans de nombreux pays. D'autre part, beaucoup de ceux qui s'en sont occupés ont surtout considéré le point de vue airographique de la question et s'en sont servis pour illustrer certaines théories du peuplement de la terre, certains centres d'origine, certaines migrations, etc... (distribution bipolaire par exemple). Or, le point de vue écoclimatologique est des plus importants : les Lichens sont parmi les meilleurs indicateurs des conditions d'humidité atmosphérique : Lichens océaniques, continentaux, steppiques, méditerranéens, etc... (voir par exemple l'étude de DEGELIUS sur l'élément océanique en Europe Occidentale). En Belgique, on peut distinguer trois zones très nettes d'humidité atmosphérique délimitées par des associations épiphytiques lichéniques : Littoral (atmosphère humide), Basse et Moyenne Belgique (atmosphère sèche), Haute Belgique (atmosphère très humide). Ce rôle d'indicateurs climatologiques joué par les Lichens n'a pas été suffisamment étudié dans les régions équatoriales et tropicales, où l'humidité atmosphérique est le facteur dominant du climat ; au Congo Belge par exemple, où de grands efforts, souvent stériles, sont faits pour dresser des cartes écoclimatologiques en fonction de la végétation supérieure, il serait facile, si des récoltes suffisantes de Lichens étaient faites en des points suffisamment nombreux, de dresser une carte des principaux types de climat en fonction de l'aire de distribution d'un certain nombre de Lichens épiphytes fruticuleux (*Usnea*, *Ramalina*, *Anaptychia*, etc...); c'est ainsi que le Bas-Congo, actuellement zone herbeuse, possède néanmoins un climax forestier dû à un haut degré

d'humidité atmosphérique, lequel est trahi par l'abondance de certaines Usnées pendant en longues barbes des branches des arbustes des savanes; l'abondance d'*Anaptychia leucomelaena*, vivant en épiphyte dans les forêts claires des plateaux peu élevés du Katanga, indique pour ces plateaux une atmosphère plus humide et plus fraîche et par là un climat plus montagnard que celui régnant dans la plaine entourant les plateaux. Il conviendrait donc d'insister pour que des récoltes de grands lichens foliacés et fruticuleux soient effectuées, même par des personnes non compétentes, dans le plus grand nombre de stations du plus grand nombre de pays possible. Les petits Lichens, spécialement les Lichens crustacés, devraient être provisoirement négligés, leur récolte et leur détermination rationnelles nécessitant généralement l'intervention de spécialistes actuellement trop rares.

3. PHYTOSOCIOLOGIE.

A. *Méthodes.* Nous ne pensons pas que les méthodes chiffrées utilisées pour relever les groupements lichéniques doivent différer de celles en usage dans l'étude phytosociologique de la végétation supérieure. La question ne se pose d'ailleurs que dans quelques cas d'associations purement lichéniques. Les estimations des degrés de recouvrement, d'abondance-dominance ou de dominance seule, de sociabilité, de fidélité, de même que la mesure de la fréquence ou de la constance, nous paraissent pouvoir être faites exactement, comme lorsqu'il s'agit de végétaux supérieurs. Certaines particularités propres aux Lichens, spécialement en ce qui concerne la sociabilité, peuvent cependant être mises en évidence par l'emploi de lettres adéquates, R signifiant par exemple une grande rosette et r une petite rosette, dans le cas d'un Lichen foliacé. Il en va de même pour l'aire minima, pour autant que l'on attache encore de l'importance à cette notion. L'évaluation de l'aire minima peut en effet se faire, aussi bien pour des associations cryptogamiques que phanérogamiques, par les méthodes courantes, et ceci même si l'on est partisan d'étudier un groupement végétal complexe, strate par strate : l'aire minima de la strate lichénique risque seulement d'être plus petite que celle de la strate arborée, tout au moins dans les cas où on considère l'aire minima comme la plus petite surface groupant une très forte proportion des espèces du groupement étudié; si l'on considère par contre l'aire minima comme la plus petite surface contenant *toutes* les espèces du groupement étudié, l'expérience nous a appris que dans ce cas l'aire minima de la strate lichénique est égale, ou parfois supérieure à l'aire minima des strates supérieures. Quoi qu'il en soit, nous ne croyons pas qu'il y ait aujourd'hui un grand intérêt (sauf dans certaines recherches particulières) à la délimitation précise de l'aire minima. Nous croyons plutôt qu'il vaut mieux délimiter sur le terrain des

surfaces où les conditions d'habitat et la végétation sont bien homogènes, et de les étudier soigneusement, quelle qu'en soit la superficie : fixer d'avance celle-ci, c'est risquer d'étudier un biotope trop petit, ou hétérogène.

Comme nous l'avons dit plus haut, nous ne croyons pas non plus qu'il convienne, lorsqu'on étudie un groupement végétal dans son ensemble, de prendre, lors du relevé phytosociologique, une plus petite surface pour la strate lichénique et muscinale que pour les strates supérieures ; les études que nous avons poursuivies dans les landes et forêts de l'Ardenne ou les maquis et garigues de la Côte d'Azur nous ont montré que, s'il faut explorer une surface d'un hectare pour détecter l'intégralité de la flore phanérogamique, cette même surface doit être prospectée si l'on désire dresser la liste complète des Lichens et Mousses formant la strate inférieure.

A titre de corollaire, il convient d'insister, auprès de ceux qui étudient les microassociations de Lichens disséminées dans une végétation supérieure, pour qu'ils tiennent compte de cette dernière et ne choisissent pas des surfaces de relevé trop petites.

B. Groupements lichéniques. Il faut à ce propos soulever une question importante : convient-il d'étudier les groupements lichéniques à part, en dehors de la végétation générale dont ils font partie ? Convient-il de décrire, en utilisant le système de Zürich-Montpellier, des associations, alliances, ordres, classes purement lichéniques, comme l'a fait par exemple KLEMENTS ? A notre avis, non. Les Lichens font partie intégrante de la végétation prise dans son ensemble et ce n'est pas parce qu'il existe des cas très spéciaux d'associations uniquement lichéniques et qui sont nécessairement des groupements absolument pionniers (Association à *Verrucaria* des roches calcaires compactes, association à *Rhizocarpon* des roches siliceuses) qu'il faille généraliser. Même dans la plupart des associations pionnières cryptogamiques des régions froides ou tempérées, les Lichens sont intimement mêlés à des Mousses, Hépatiques, Algues, Cyanophycées et Champignons, voire même à des Phanérogames (exemples du *Corynephorion canescentis* en Europe atlantique, de l'*Helianthemion guttati* en Europe méditerranéenne). Les séparer de ces associés en des groupements lichéniques à allure homogène est un erreur écologique et phytosociologique.

Lorsqu'il s'agit de la strate au sol de groupements phanérogamiques développés (landes, pelouses, forêts, maquis, garigues, etc...), la situation est encore plus nette : il n'est plus possible de considérer des associations lichéniques indépendantes (Exemple : *Cladonia mediterranea*, *Cl. verticillata*, *Cornicularia aculeata*, etc... très fidèles caractéristiques des landes siliceuses de l'ordre des *Lavanduletalia* en Méditerranée française).

Dans les régions tropicales, l'intrication des groupes systématiques est

en général plus complète que dans les régions tempérées, et il devient souvent illusoire d'y délimiter des associations lichéniques.

Nous croyons donc qu'il convient d'intégrer les Lichens dans les groupements végétaux généraux dont il ne sont souvent que les composants d'une strate; il n'y a pas lieu de faire un système à part des groupements lichéniques, ceux-ci s'intégrant aisément dans le système général et unique des unités phytosociologiques du globe terrestre.

C. *Prodrome*. Selon les considérations énumérées ci-dessus, l'élaboration d'un prodrome des associations lichéniques ne nous paraît ni heureuse ni nécessaire. Les Associations lichéniques, même composées uniquement de Lichens, ont leur place dans le Prodrome général des Associations végétales (voir à ce sujet le système proposé par RUEBEL, *Planzengesellschaften der Erde*, 1930). Il serait plus utile d'élaborer un Prodrome des groupes écologiques de Lichens, comme l'a fait DES ABBAYES dans son traité (exemples: Lichens des troncs des jeunes arbres à écorce lisse, Lichens des rochers siliceux moussus, Lichens des landes à Bruyères, Lichens des terres calcaires, Lichens des troncs pourrissants, etc...). Ces groupes pourraient alors être intercalés comme indicateurs écologiques importants dans les associations végétales dont ils font partie intégrante.

Dans le Prodrome proposé, on pourrait reprendre les données de la phytosociologie générale en même temps que celles de la phytosociologie particulière des Lichens et on y rassemblerait également les données de la phytogéographie et de l'écologie.

Ce Prodrome comporterait deux parties:

1. Aperçu des éléments phytogéographiques.
2. Aperçu des groupes écologiques.

Dans le Prodrome général des associations végétales du globe terrestre, il va sans dire que certains chapitres seraient réservés soit aux Unités supérieures purement lichéniques, comme les *Verrucarietalia* ou les *Rhizocarpetalia*, soit aux Unités surtout Cryptogamiques où abondent les Lichens, comme par exemple la Classe des Associations épiphytiques des régions tempérées de l'Europe occidentale; dans ce dernier cas, les Bryophytes, Algues et Cyanophycées sont inséparables des Lichens.

Des exemples concrets de ce qui vient d'être exposé seront présentés lors des séances du Congrès de Paris.

DIE FLECHTEN DER TROPEN

von Fritz MATTICK

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RAPPORT

1. *Geschichte und Literatur.* — Unsere Kenntnis der tropischen Flechten beginnt mit einigen wenigen Arten, die schon in der ersten Epoche der Lichenologie bekannt wurden, in der vorlinneschen Zeit. J. PETIVER erwähnt 1695 zwei tropische Parmelien, H. SLOANE zählt 1696 7 Arten aus Jamaica auf, J. DILLENUS 1741 weitere aus Ostindien, St. Helena und Madagaskar. — Nach 1753, dem Beginn der zweiten lichenologischen Epoche, setzt verstärkte Erforschung der Tropen ein, besonders mit den Werken von O. SWARTZ, der in mehreren Arbeiten von 1788 bis 1811 viele Arten aus Westindien, besonders Jamaica, beschreibt. Aus Indochina führt J. DE LOUREIRO 1793 9 Arten auf. 1803 folgt BORY DE ST. VINCENT mit Flechten aus Mauritius, 1810 PERSOON mit solchen aus S. Domingo, und A. L. FÉE erwähnt 1824 nicht weniger als 336 tropische Arten. Rasch steigert sich die Zahl der tropischen Flechten ebenso wie die der Autoren. Gegen Ende dieser Epoche gilt NYLANDER als bester Kenner exotischer Flechten; von den 1348 Arten, die ihm 1857 bekannt sind, gehört eine beträchtliche Anzahl den Tropen an. Auf ihre Aufzählung sowie die der Autoren kann ich hier verzichten, da KREMPELHUBER (1867-72) sie genauestens verzeichnet hat. Es sei aber zusammenfassend erwähnt, dass von den 1813 bis zum Jahre 1870 überhaupt erschienenen lichenologischen Arbeiten 93 (also 5 %) tropische Flechten behandeln; davon entfallen auf die tropischen Gebiete Afrikas 8, Amerikas 43, Asiens 13 und der Südsee 11. 8 berichten über die Ausbeute der grossen Schiffsexpeditionen, 10 behandeln allgemein exotische Flechten.

1870 beginnt mit der Erkenntnis des symbiotischen Wesens der Flechten die dritte Epoche der Lichenologie. In dieser erreicht die Zahl der Schriften über tropische Flechten das vierfache, nämlich 400 von 6.000 seitdem erschienenen lichenologischen Arbeiten; der prozentuale Anteil steigt aber nur wenig, nämlich von 5 % auf 6,6 %. 192 Autoren schreiben in dieser Zeit über tropische Flechten. Von ihnen kann ich hier nur die allerbedeutendsten nennen, nämlich nochmals NYLANDER mit 20 weiteren Arbeiten aus der Zeit von 1874-1900, J. MUELLER-ARG. 55 (1879-96), E. WAINIO 27 (1890-1929), A. ZAHLBRUCKNER 26 (1896-1943) und G. MALME 14 (1897-1937). Weitere bedeutende Autoren nenne ich in einer ausführlicheren Arbeit über das gleiche Thema, die in den Berichten der Deutschen Botanischen Gesellschaft erscheinen wird. Ueber Afrika handeln 109 Ar-

beiten, Asien 76, Nord- und Mittelamerika 70, Südamerika 69, Australien und Südsee 41 (immer sind nur die tropischen Gebiete gemeint, also Südastralien/Neuseeland, Kapland usw. weggelassen). Ich möchte betonen, dass hierbei nur solche Arbeiten berücksichtigt sind, die im Titel ein bestimmtes tropisches Gebiet nennen; weit erhöhen würden sich die Zahlen, wenn auch die vielen Veröffentlichungen hinzugerechnet würden, die zwar auch Beschreibungen tropischer Arten bieten, sich aber nur allgemein « Neue Flechtenarten », « Lichenologische Notizen » oder ähnlich nennen, so dass man aus dem Titel nichts Genaueres ersehen kann. Im ganzen ergeben sich somit rund 500 Arbeiten über tropische Gebiete gegenüber 7.300 über aussertropische.

2. *Grad der lichenologischen Erforschung der Tropen.* — Wenn wir bedenken, dass die tropischen Landgebiete der Erde mit 69 Millionen Quadratkilometern 46 % der Landmasse ausmachen gegenüber den 80 Millionen Quadratkilometern, d.h. 54 % der gemässigten und kalten Zonen, so müssten den 7.300 lichenologischen Arbeiten über die gemässigten und kalten Gebiete 6.400 über tropische gegenüberstehen. Da in Wirklichkeit aber erst 500 Veröffentlichungen die Flechten der Tropen behandeln, sind dies nur 8 %, oder mit anderen Worten, unsere Kenntnisse über tropische Flechten betragen an Intensität erst 1/12 dessen, was wir über die Flechten der gemässigten und kalten Gebiete wissen. Und da selbst dort nur wenige und kleine Landstriche (nämlich in den dicht besiedelten Gebieten) gründlich, die meisten noch recht mangelhaft lichenologisch erforscht sind, kann man von den Tropen sagen, dass ihre Durchforschung erst ganz am Anfang steht. Alle Angaben und Vergleiche, die wir über die Tropenflechten machen können, sind also mit grösster Vorsicht zu geniessen. Eine Karte der ganzen Erde, in der durch Punkte alle Gebiete eingetragen sind, in denen bisher Flechten in grösserer Menge gesammelt wurden, hat noch niemand gezeichnet; sie würde das erschreckend verdeutlichen! Eine kleine Ahnung davon gibt die SANDSTEDESche ganz allgemein gehaltene Karte (*Pflanzenareale* 1938).

Die meisten Arbeiten sind nur listenmässige, floristische Aufzählungen der Arten bestimmter Gebiete. Bedenkt man, dass manche Autoren tropische Flechten bearbeitet haben, obwohl sie wegen mangelnder Literatur- und Herbar-Vergleichsmöglichkeiten gar nicht in der Lage waren, genaue Bestimmungen vorzunehmen, und dass selbst die berühmtesten Lichenologen von anderen Autoren schon beschriebene Arten (oder sogar ihre eigenen früheren) nochmals unter anderem Namen als neu beschrieben, so erkennt man, wie kritisch man an alles Vorhandene herangehen muss. Die meisten Arbeiten über tropische Gebiete beruhen auf Material, das von Nicht-Lichenologen oft nur zufällig gesammelt wurde. Nur ganz wenigen Lichenologen, wie WAINIO und MALME, war es vergönnt, selbst in den Tropen

zu sammeln und auf Dinge zu achten, die spezielles Interesse verdienen und einem Nicht-Lichenologen leicht entgehen.

3. *Floren*. — Auch innerhalb dieser dünnen Gesamt-Intensität der lichenologischen Bearbeitung tropischer Gebiete ist wieder die Verteilung recht verschieden. Einige Gebiete sind relativ etwas besser berücksichtigt worden, so z.B. die Antillen mit 50, Brasilien mit 64, Indonesien mit 32, die Südsee, besonders Hawaii, mit 46 Arbeiten. Floren oder wenigstens umfassendere Uebersichten von Einzelgebieten existieren erst ganz wenige. WAINIO schrieb solche für das von ihm bereiste Gebiet Brasiliens und für die Philippinen, MALME mit seinen Mitarbeitern für sein südamerikanisches Reisegebiet, ZAHLBRUCKNER-MAGNUSSON für Hawaii, ZAHLBRUCKNER für Java. Eine Flechtenflora für ganz Brasilien hatte ZAHLBRUCKNER geschrieben, aber das Manuskript ist durch den Krieg vernichtet worden. — Selbst nur nach der bisher vorliegenden Literatur zusammengestellte Namenslisten sind schon nützlich, wie sie kürzlich DODGE für Südafrika und BISWAS für Indien veröffentlicht haben. — Dringend nötig wäre aber einmal ein Abbildungs- und Bestimmungswerk über die wichtigsten, allgemein verbreiteten tropischen Laub- und Strauchflechten (etwa ähnlich dem ANDERS-chen für Mitteleuropa), das demjenigen, der sich für die Tropen interessiert, wenigstens eine erste Hilfe und Einführung sein könnte. VERDOORN hat schon auf dem Kongress 1950 bedauert, dass es niemand möglich ist, der in den vorhandenen biologischen Stationen der Tropen arbeiten wil, eine Uebersicht der dort anzutreffenden häufigsten Kryptogamen vorzufinden.

Immerhin lassen sich schon verschiedene Florengebiete in grossen Zügen abgrenzen. So weist DODGE (1953) darauf hin, dass Tropisch-Westafrika kaum Beziehungen zu Nordafrika hat und dass das abessinische Hochland deutlich vom Kenya-Gebiet getrennt ist, wie sich auch das westliche vom östlichen Zentralafrika unterscheidet. Ebenso lässt sich Südafrika abtrennen, und entsprechend dürften Westindien, die Andengebiete, das brasilianische Küstengebiet, die nordost-brasilianische Trockenregion, Ostindien, Malesien und Polynesien besondere Florengebiete darstellen. Andererseits sind auch verbindende Züge zu finden, so nach DES ABBAYES zwischen Westafrika und Südamerika, zwischen Madagaskar und Indonesien-Ozeanien.

4. *Verbreitung der Taxa*. — Wie ist nun die Verteilung der bisher bekannt gewordenen Flechten über den Tropengürtel? Von den bis heute überhaupt beschriebenen Flechtenarten, die sich auf rund 18.000 belaufen, mögen etwa 10.000 allein oder überwiegend in den Tropen vorkommen. Das ist etwa ein Zehntel der höheren Pflanzen der Tropenzone, so dass diese einen Flechten-Koeffizienten von 0,1 aufweist. Wie ich 1953 gezeigt habe, wird der Flechten-Koeffizient über die gemässigten noch den kalten Zonen immer grösser, da die Flechten nach den Polen hin eine immer

grössere Bedeutung in der Gesamtflora erlangen. Natürlich schwankt der FK auch innerhalb der Tropenzone je nach den örtlichen Gegebenheiten sehr, beträgt z.B. für Java (600 Flechtenarten) 0,06, für Brasilien (2-3000 Flechten) 0,1, für die Hawaii-Inseln (500 Flechten) schon 0,42.

Die Bedeutung der einzelnen Flechtenfamilien für die Tropen ist recht verschieden. Manche in den gemässigten und kalten Zonen artenreichen Familien fehlen in den Tropen fast ganz, wie die *Verrucariaceae*, *Dermatocarpaceae*, *Umbilicariaceae*, andere sind zwar vorhanden, aber nicht so reich entwickelt wie ausserhalb der Tropen, z.B. *Lecanactineaceae*, *Gyalectaceae*, *Pyrenopsidaceae*, *Lichinaceae*, *Peltigeraceae*, *Acarosporaceae*. Wieder andere mögen etwa gleichmässig über die verschiedenen Zonen verteilt sein, wie die *Pyrenulaceae*, *Caliciales*, *Arthoniaceae*, *Collemataceae*, *Lecideaceae*, *Cladoniaceae*, *Lecanorales* und *Caloplacales*. Andere endlich überwiegen in den Tropen, wie die *Graphidaceae*, *Chiocetaceae*, *Thelotremaaceae*, *Pannariaceae*, *Stictaceae*, oder sind völlig auf die Tropen beschränkt, wie die *Trypetheliaceae*, *Paratheliaceae*, *Astrotheliaceae*, *Strigulaceae*, *Cryptotheciaceae*, *Roccellaceae*, *Chrysotrichaceae*, *Asterothyriaceae*, *Phyllopsoraceae* und die *Basidiolichenes*. Mehr als 60 tropische Gattungen sind bisher überhaupt nur mit einer Art und aus nur einem bestimmten Gebiet bekannt. Aus sonst weiter verbreiteten Familien sind manche Gattungen ganz oder überwiegend auf die Tropen beschränkt, wie *Coccocarpia*, *Erioderma*, *Lobaria*, *Sticta*, *Megalospora*, *Lopadium*, *Bombyliospora*, *Teloschistes* und *Pyxine*. — Für die Verbreitung einzelner Gattungen mögen nur vier Beispiele genannt werden: Von 271 *Cladonia*-Arten sind 162 vorwiegend aussertropisch, 109 tropisch, davon aber nur 27 über weitere Gebiete der Tropen verbreitet, die übrigen auf Einzelgebiete beschränkt, z.B. 48 auf das tropische Amerika, die Untergattung *Clathrina* vorwiegend auf die südliche Halbkugel. — Umgekehrt überwiegt *Sticta* (in dem 4 Untergattungen umfassenden weitesten Sinne) mit 126 tropischen Arten gegenüber 103 aussertropischen; auch hier wieder ist das tropische Amerika mit 40 Arten vor den übrigen Kontinenten führend, und das tropische und warm-gemässigte Gebiet von Südamerika bis Neuseeland-Australien umschliesst das Verbreitungszentrum. — *Usnea* hat 191 aussertropische gegenüber 260 tropischen Arten; von diesen sind ebenfalls nur 24 über weitere Bezirke der Tropen verbreitet, von den übrigen 94 auf die Tropengebiete Amerikas, 75 Afrikas, 47 Asiens und 20 Ozeaniens beschränkt. Auch die einzelnen Unterabteilungen dieser Gattung weisen nach den Angaben MOTYKAs deutlich geographische Zentren auf, so ist die Untergattung *Eumitria* überwiegend tropisch, und von *Euusnea* kommen die Sektionen *Glabratae* in den Tropen, *Stramineae* und *Dendriticae* in den Tropen der Südhalbkugel, *Articulatae* hauptsächlich in tropischen Afrika und *Laevigatae* vor allem in Südamerika vor. — Die Gattung *Parmelia* endlich hat 311 vorwiegend aussertropische Arten gegen-

über 402 tropischen. Auch hier sind nur recht wenige weiter in den Tropen verbreitet, nämlich 36, während das tropische Amerika, vor allem Brasilien, mit 205 einen ganz besonders hohen Artenreichtum aufweist gegenüber 87 tropisch-afrikanischen, 59 asiatischen und 15 ozeanischen. Hier bevorzugen gleichfalls manche Untereinheiten besonders deutlich die Tropen, wie *Xanthoparmelia* und *Amphigymnia*. — Ob bei allen genannten Gattungen der besondere Artenreichtum tropisch Amerikas Tatsache ist oder auf der besseren Durchforschung beruht, muss die Zukunft zeigen. Jedenfalls hat auch *Menegazzia* deutlich ihr Zentrum in Südamerika (SANTESSON 1942). — So kann man hinsichtlich der Flechtenflora der Tropen schon jetzt von deutlichen Entstehungszentren und Florenelementen sprechen. Wieweit nun auch klimatisch bedingte Gruppierungen, z.B. ozeanische Arten (beispielsweise bei den *Roccellaceae*) gegenüber kontinentalen Arten, Tieflands- und Hochgebirgsarten zu unterscheiden sind, ist ebenfalls noch näher zu untersuchen.

Bei vielen Arten, die früher für Kosmopoliten gehalten und auch für die Tropen angegeben wurden, hat sich bei genauerer Untersuchung herausgestellt, dass sie aufzuteilen sind und dass die eine Art neuen Umfangs in einem anderen Florengebiet durch eine oder mehrere « vikariierende » ersetzt wird, so z.B. nach SANTESSON 1942 *Cladonia rangiferina* im gemässigten Südamerika durch *Cl. vicaria*, *Cl. mitis* durch *Cl. laevigata*, und entsprechend in den Tropen Südamerikas *Cladonia impexa* durch *Cl. confusa*, *pyncoclada*, *pohlia*, *sandstedei* und *signata*. MOTYKA hat Ähnliches für *Usnea*-Arten nachgewiesen, und entsprechend werden sich in Zukunft noch viele andere Fälle aufspüren lassen.

Ein weiteres interessantes Problem, das auch die Tropen berührt, ist das der bipolaren Taxa, d.h. solcher Einheiten, die in den gemässigten oder kalten Gebieten beider Halbkugeln vorkommen. Ihre Nord- und Süd-Areale müssen früher über die Tropen hinweg in Verbindung gestanden haben oder tun es heute noch über die Höhenstufen der tropischen Gebirge. Diese dienten ihnen als Brücken für ihre früheren Wanderungen, und dort finden sie noch heute zusagende Klimabedingungen. DU RIETZ hat 1940 schöne Beispiele dafür gezeigt und vor allem die Anden als Wanderungswege aufgeführt, z.B. für die *Usnea*-Untergattung *Neuropogon*.

Im Gegensatz zu den vielen floristischen Arbeiten über bestimmte Tropengebiete liegen nur ganz wenige vor, die sich die monographische Bearbeitung einer bestimmten Gruppe oder Gattung vorgenommen haben (etwa 30); besonderes Interesse haben dabei die *Basidiolichenes* gefunden (12).

5. *Phylogenie*. — Da paläolichenologische Funde nicht vorliegen, können wir unsere Schlüsse über die stammesgeschichtliche Entwicklung der Flechten nur aus der Organisationshöhe ihrer heute lebenden Vertreter ziehen. Vieles spricht dafür, dass die Tropen die Urheimat der Flechten

darstellen und dass die symbiotische Verbindung baumbewohnender Pilze mit rindenbewohnenden Luftalgen, erst zufällig und locker, später in festerer Gemeinschaft, hier zur polyphyletischen Entstehung und weiteren Entwicklung dieser Pflanzengruppe geführt hat. In den Tiefländern der Tropen dürften sich die klimatischen und sonstigen Umweltsverhältnisse seit langen Zeiten wenig verändert und daher auch für manche alten und weniger hoch organisierten Flechtengruppen nur geringer Anreiz zur Höherentwicklung vorgelegen haben. Solche Gruppen, wie die *Pyrenulales* und *Graphidales*, spielen daher eine besondere Rolle in der Flechtenflora der Tropen, nicht zum mindesten auch deshalb, weil die ihnen zusagenden Substrate, die Baumrinden und Blätter, oft die einzigen in den Flachländern dieser Breitengrade zur Verfügung stehenden sind. — Der Anstoss zur Fortentwicklung, der durch die Auftürmung der tertiären Faltengebirge und die damit verbundenen Klimaänderungen in den höheren Breiten zur weiteren Entfaltung vieler Flechtentaxa geführt hat, wird sich in geringerem Masse auch in den Gebirgsgebieten der Tropen bemerkbar gemacht haben, und welche Rolle diese Gebirge für frühere Pflanzenwanderungen, auch der Flechten, gespielt haben, wurde schon erwähnt. — Andererseits scheint sich noch heute mehr als in anderen Gebieten gerade in den Tropen die Ausbildung und Entwicklung neuer Einheiten fortzusetzen, wie das nur hier verwirklichte und zuweilen recht beachtliche Vorkommen der *Basidiolichenes* zeigt. Diese weisen in manchen Formen noch recht wenig eigentliche Flechtencharaktere auf; die lichenisierten Clavarien und häufig zu beobachtenden « Halbflechten » stellen erst Anfänge einer Flechtenwerdung dar. — Auch das häufige Vorkommen von Luftalgen (besonders Trentepohlien) in den verschiedensten Stufen der Selbständigkeit bis zur festen Lichenisierung mit Pilzen, ist hier zu erwähnen (s. MATTICK 1953).

6. *Biologie und Ökologie*. — Erst ganz wenig Literatur existiert über die biologischen Verhältnisse der Tropenflechten. Einige Untersuchungen über Wasseraufnahme und Atemöffnungen hat GOEBEL in Buitenzorg angestellt. Besonderer Beliebtheit hat sich das Problem der epiphyllen Flechten erfreut, das seit 1884 in 11 Arbeiten behandelt und neuerdings von SANTESSON (1952) erschöpfend dargestellt wurde. — Im übrigen aber ist die Abhängigkeit der Flechten von den Klima — und Substratverhältnissen überhaupt noch nicht untersucht worden. Man müsste z. B. feststellen, bei welcher Intensität die Verträglichkeitsgrenzen gegenüber Hitze und Trockenheit, Licht und Beschattung, verschiedenartiger Reaktion des Substrates usw. liegen, ob sich unter dem Einfluss dieser Faktoren Licht-, Schatten- und andere anatomisch oder physiologisch unterscheidbare Standortformen ausbilden können, wie die Wachstumsgeschwindigkeiten der tropischen Flechten sind und ob man Jugend- und Altersformen unterscheiden kann, welche Konkurrenzkämpfe unter den Arten gleichen Standortes sich abspielen, und was dergleichen Fragen mehr sind. Schon

jetzt zeigt einfache Beobachtung, dass etwa *Coenogonium* besonders geringe Licht- und starke Feuchtigkeitsansprüche stellt, dass andererseits *Caloplaca*-Arten der trockenheissen Savannen grösste Hitze, Trockenheit und Belichtung aushalten oder die felsbewohnenden Usneen und Umbilicarien der Hochgebirgsstufen auch ziemlich Kälte ertragen können. — Leiter bietet die bisherige Literatur fast keinerlei derartige Angaben, im günstigsten Falle nennt sie wenigstens das Substrat. Auch über die Veränderungen der Flechtenflora entsprechend den Klimawandlungen in den Höhenstufen der Tropengebirge liegen erst ganz spärliche Beobachtungen vor.

7. *Soziologie*. — Der gleiche Mangel der vorliegenden Literatur wird auch in Hinblick auf soziologische Fragen ersichtlich. So zeigt sich beispielsweise selbst ein so wichtiges Werk wie das von WAINIO über Brasilien doch als an seine fast ausschliesslich systematisch-floristisch interessierte Zeit gebunden. Es stellt nur eine Florenliste dar und gibt keinerlei Aufschluss darüber, wie nun die Flechtenwelt dieses Gebietes wirklich aussieht, welche Arten tonangebend in der Vegetation sind oder gar das Landschaftsbild beherrschen (wie etwa die prachtvollen, ausgedehnten *Cladonia*-Rasen auf den Höhen des von ihm so eingehend untersuchten Carassa-Gebirges), wie sich die verschiedenen Arten zu Gesellschaften zusammenfinden und wie sie von ihrer Umwelt abhängig sind. Es gibt auch heute noch keine Arbeiten, die Flechtengesellschaften der Tropen behandeln. Das hat natürlich seinen Grund darin, dass man dazu erst einmal die Arten kennen muss, dass aber für die meisten Tropengebiete noch nicht einmal Artenlisten vorliegen. Man kann nicht, wie etwa in Skandinavien oder Deutschland, mit vorheriger Kenntnis wenigstens der wichtigeren zu erwartenden Arten sich ins Gelände begeben und dort soziologische Aufnahmen machen, sondern man sieht sich meist unbekannten Arten gegenüber, die erst in der Heimat und mit Hilfe von Spezialisten bestimmt werden können.

Und doch kann man, wie mir meine eigenen Beobachtungen in Brasilien zeigten, auch in den Tropen gut charakterisierte und deutlich von einander abgegrenzte Flechtengesellschaften feststellen. Typische Gesteins-, Erd- oder Rindengesellschaften sind überall anzutreffen, z. B. die Fels-Usneen-Bestände höherer Lagen im Gebirge, die *Caloplaca-Parmelia*-Gesellschaften auf den Gesteinsflächen der Caatinga. Deutliche Parallelen lassen sich erkennen mit Gesellschaften, die in anderen Breiten unter ähnlichen ökologischen Bedingungen leben, z.B. zwischen den *Sticta*-Assoziationen des Regenwaldes und den Lobarieten unserer Buchenwälder oder zwischen der *Cladonia aggregata* — Gesellschaft auf dem Sandboden der Restinga-Strandwälder und den *Cornicularia* — Heiden nordwestdeutscher Dünen; man kann hier von « vikariierenden Gesellschaften » reden. Viele solche tropischen Gesellschaften lassen sich ohne weiteres in das soziologische System (wie ich es 1951 aufgestellt habe) einreihen; für andere ist es durch neue Einheiten zu erweitern.

8. *Angewandte Lichenologie*. — Wie weit Flechten tropischer Gebiete von deren Bewohnern zum Färben von Fasern und Stoffen, als zusätzliche Nahrungsmittel, als Gifte oder anderweitig verwendet werden, ist uns noch nicht bekannt. Einige indische und brasilianische Arbeiten haben sich in den letzten Jahren der chemischen Erforschung von Flechtenstoffen tropischer Arten gewidmet; betreffs antibiotischer Wirkung von Flechtenstoffen liegen noch keine Untersuchungen aus den Tropen vor.

9. *Methodik und Organisation*. — Die vorstehenden Ausführungen zeigen, wie unendlich viel in den Tropen noch auf allen Gebieten der Lichenologie zu tun ist. Dies könnte, vor allem hinsichtlich solcher Aufgaben, bei denen es nicht nur auf das Einsammeln von Material ankommt, sondern für die eine möglichst langdauernde Anwesenheit am gleichen Ort erforderlich ist, natürlich am besten durch einheimische Lichenologen geschehen; aber solche gibt es in den Tropenländern noch recht wenige, etwa in Brasilien und Indonesien. Biologische Forschungsstationen, die als Basis dienen können, müssten in noch grösserer Zahl gegründet werden, besonders in den abwechslungsreichen Hochgebirgen. Wie bedauerlich ist es, dass heutzutage Hochgebirgsexpeditionen in den Tropen meist nur rein bergsteigerische Ziele haben und dass für wissenschaftliche Ausrüstung nicht die genügenden Gelder aufgetrieben werden können. Was könnte eine Expedition, die etwa auch mit einem Hubschrauber (Helicopter) ausgerüstet wäre, an aufschlussreichen Beobachtungen und wertvollstem Material beispielsweise von den tropisch-afrikanischen Bergen, aus den Anden, den Hochgebirgen von Madagaskar und Neuguinea mitbringen! Uebernationalen Organisationen (etwa die UNESCO) müssten hier helfen, um die Durchführung solcher Forschungsarbeiten zu erleichtern, die sonst wegen der hohen mit einer Tropenreise verbundenen Kosten den meisten daran interessierten Fachleuten durchzuführen unmöglich sind.

Erwähnte Schriften, in denen weitere *Literatur* zitiert ist:

MATTICK (F.). — Wuchs- und Lebensformen, Bestand- und Gesellschaftsbildung der Flechten. — *Botan Jahrbücher*, 75, 1951, 378-424. — Alte und neue Probleme der Lichenologie. — *Ber. Deutsch. Bot. Ges.*, 64, 1951, 93-107. — Lichenologische Notizen, 1-5, *Ibid.*, 66, 1953, 263-276.

Résumé. — Un coup d'œil sur l'histoire des recherches lichénologiques dans les pays tropicaux fait le point de nos connaissances actuelles. La flore existant dans ces régions est dénombrée et la répartition des unités systématiques les plus importantes est décrite, notamment la limite des espèces proprement tropicales et de celles qui ont une signification phylogénétique. Une vue d'ensemble des nombreux problèmes non résolus, surtout d'ordre biologique, écologique et sociologique, montre l'ampleur des recherches restant encore à effectuer.

COMMUNICATIONS DIVERSES

ON THE ACTION OF LICHEN ACIDS
ON CARBOHYDRATE METABOLISM

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Experiments have been performed in order to study the effects of organic acids from lichens on metabolism of animals as well as of baker's yeast. Particularly interesting results have been obtained with vulpinic acid (well known from *Letharia vulpina* (L.) Vain.) and usnic acid (obtainable from most members of the genus *Cladonia* (Hill.) Vain.).

Both acids give in a dose of 10 mg/kg body weight, administered intravenously as sodium salt, a prompt and heavy rise in oxygen consumption accompanied by a pronounced increase in rate and amplitude of respiration and by a gradual rise in body temperature, lasting for about 5 hours and then slowly falling.

Larger doses than 10 to 30 mg/kg are generally lethal. In some occasions the hyperthermia probably has been the direct cause of death. In a few cases the rectal temperature has exceeded 45° C.

As a rule lethal doses of usnic acid cause an intense rigor mortis. This effect is similar to the well known action of dinitrophenols.

In normal narcotized cats an injection of sodium usnate is followed by a steep rise in arterial blood sugar to a level of 300-400 mg % lasting for about the same time as the increased respiratory volume. In fasted cats the rise is much slower and somewhat smaller. It is concluded that the major part of the rise in arterial blood sugar is due to an increased breakdown of glycogen to glucose, but there may be increased gluconeogenesis.

Whereas the effects of usnic acid in the cat are very similar to those of the dinitrophenols, vulpinic acid influences metabolism in a somewhat different way because intoxication does not produce rigor mortis nor does the acid possess a toxic effect on a diabetic organism. In modest doses it may, on the contrary, prevent glucosuria.

In baker's yeast both substances induce a considerable endogenous fermentation whereas the fermentation of sugars is markedly depressed. The inhibition of the fermentative breakdown of « adaptive » sugars such as maltose and galactose is particularly pronounced.

PAPERCHROMATOGRAPHIC METHODS IN LICHEN CHEMISTRY

by Carl Axel WACHTMEISTER

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The method of paper chromatography seems to offer great possibilities in the field of lichen chemistry, especially with regard to taxonomical problems. The lichen acids may conveniently be chromatographed on paper strips impregnated with phosphate buffer. Spots of aromatic acids are detectable in ultra-violet light and may be further characterized by the colours obtained with several reagents, e. g. diazotised benzidine. Thus, the acetone extract of a small sample of lichen will suffice for obtaining a « chemical finger-print » of the species or variety. In this way, the chromatograms may be of a certain taxonomical value even without a detailed chemical interpretation. However, their full value is realized only when the spots are identified by chromatographic comparison with pure reference substances.

Prior to a more thorough chromatographic study of lichens belonging to the genus *Pertusaria* and *Haematomma*, we have started to elucidate the structures of some unknown acids. E. g., ocellatic acid from *P. coralina* (Ach.) have proved to be identical with thamnolic acid. Porphyrilic acid from *Haematomma coccineum* (Dicks.) has been shown to be a derivative of the unsymmetrical 1,7-dihydroxy-dibenzofuran, in contrast to strepsilin and didymic acid, which are symmetrical derivatives of dibenzofuran.

An investigation, partly by chromatographic methods, of the low molecular carbohydrate constituents of a number of lichens has revealed some regularities of a certain taxonomical interest.

A PROPOSED PROGRAM OF COOPERATIVE MONOGRAPHIC ATTACK ON THE LARGER LICHEN GENERA

by Iván MACKENZIE LAMB

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A scheme is proposed for the monographic treatment of the larger lichen genera by the cooperative international effort of a team of specialists working in unison according to previously standardized principles, and

sending their results on specially prepared punch-cards, which would be stored and processed in a central institution. It is calculated that a team of ten or twelve specialists, working simultaneously along these standardized lines, and evaluating the results obtained by the application of mechanical sorting methods, could accomplish the adequate monographic treatment of one of the larger lichen genera, such as *Lecanora* or *Lecidea*, in three to four years, a work which would take more than the whole lifetime of a single specialist working unaided.

PROJET DE CONSTITUTION D'UNE SOCIÉTÉ D'ÉCHANGES DE LICHENS

par Y. RONDON

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Cette société pourrait être conçue, à l'échelle internationale, sous la forme des sociétés similaires existant déjà. Nombre de membres limité à 20. Nouvelles adhésions reçues dans la limite des membres à remplacer. Chaque membre s'engage à fournir annuellement un minimum de 10 espèces ou variétés, présentant un intérêt réel, à l'exclusion des banales, en 20 exemplaires semblables et provenant de la même localité. Chaque échantillon de la même espèce ou variété sera présenté en sachet et accompagné d'une étiquette avec numéro de référence, nom, localité, etc. L'envoi en sera fait à un secrétaire à désigner, qui en assurera la répartition. Les frais de répartition et de correspondance seront couverts par une cotisation.

OBSERVATIONS SUR LES LICHENS CORTICOLES DE LA FORÊT DE ST-GERMAIN-L'HERM (PUY-DE-DOME)

par L. BERNER

Marseille

La Sapinière (altit. 1.075 m.) est traversée par la route entre Chaise Dieu et Issoire, formant une lisière sur le plateau. Aux branches nous avons récolté les 3/5 et 30/9 1952 les lichens scia-hygrophiles :

- a, b, c, d, e: *Alectoria jubata* (L.) Ach. (var. *sorediata*) — commun
- a, b, c, d, e: *Cetraria glauca* (L.) Ach.
- a, b, c, d, e: *Evernia prunastri* (L.) Ach.
- b, c, d, : *Letharia divaricata* (L.) Hue — commun
- a, b, c, d, e: *Parmelia furfuracea* (L.) Ach.
- a, b, c, d, e: — *physodes* (L.) Ach.
- a, b, c, d, e: — *sulcata* Tayl. (var. *minor*)
- c, d, : *Phlyctis agelaea* (Ach.) Kærst.
- b, c, d, e: *Ramalina farinacea* Ach. — commun
- b, c, d, e: *Usnea ceratina* Ach.
- b, c, d, e: — *dasyopoga* (Ach.) Nyl. — très commun
- a, b, c, d, : — *florida* (L.) Hoffm. — commun
- *plicata* (L.) Fries
- *scabrata* Nyl.

Les espèces précédées de : « a » figurent dans *Usnea florida communities* d'ALMBORN (1948) (1) sur les 14 citées aux branches de hêtres en Scandinavie mérid. ; de « b » sont au Mont-Ventoux (Vaucluse) sur Pins (altit. 11-1400 m.) selon RONDON (1951) et de « c » à la Ste-Baume près Marseille sur hêtres et pins (altit. 7-900 m.), de « d » en Basse-Provence sur pins (altit. 3-900 m.) selon DUGHI et DUCOS (1938), enfin « e » marque les lichens de préférence corticoles aux étages inférieurs et saxicoles à l'étage alpin.

Comme il y a plus de lumière et d'humidité en haut du tronc chez les feuillus et de meilleurs facteurs écologiques à la base des résineux, nous ne nous arrêtons pas sur l'implantation. Mais avec SMITH (1921) nous concluons qu'aucun substratum ne caractérise un Lichen dont la distribution est fonction du climat ou de la nature du sol pour certains genres dans l'aire.

Par suite de larges aptitudes adaptatives, les Lichens d'exigences écologiques assez similaires peuplent bien un milieu déterminé, mais à titre de collection, sans former un ensemble, les individus demeurant indépendants. *Usnea florida* chez ALMBORN (bien que plus répandu en France qu'ailleurs en Europe, Asie Mineure et Amérique Septentrionale selon MOTYKA [1936/8]) cède la dominance à *dasyopoga* dans le Livradois, l'altitude compensant la latitude pour les facteurs climatiques; au Mont-Ventoux il y a équivalence entre *florida* et *ceratina*, cette dernière demeurant seule à 900 m. sur Cèdres et à la Ste-Baume *florida* est rare, toujours avec le cortège mentionné.

Donc les Lichens s'installent selon leurs exigences écologiques, sans réaction commune ou collective pour constituer un groupement quelconque. Certains sont plus rapides ou ont plus de facilités pour occuper un emplacement convenable, notamment des stations créées par l'Homme, mais

(1) Les autres se rencontrent aussi en Scandinavie.

c'est toujours la course pour la meilleure place, l'élimination par le plus apte, sinon un concours de circonstances fortuites qui marquent un peuplement lichénique.

Ouvrages cités : — ALMBORN (O.). - Distribut. and Ecology of some south. scand. Lichens, *Bot. Not.* suppl. vol. 1, Lund 1948. — DUGHI (R.) et DUCOS (F.). - Les Lichens pinicoles en Basse-Provence, *Ann. Fac. Sc.*, XI, Marseille 1938. — MOTYKA (J.). - *Lichenum gen. Usnea. Stud. monogr.*, Leopoli 1936/8. — RONDON (Y.). - Une station de Lichens pinicoles au Mont-Ventoux, *Feuille d. Naturalistes N. S.* VI, Paris 1951. — SMITH (A.-L.). - British Lichens, London 1921.

SECTION 19

MYCOLOGIE

I. Mycologie générale

1. — BASES DE LA SYSTEMATIQUE CHEZ LES PYRENOAMYCETES

RAPPORT

THE BASES OF THE SYSTEMATIC RELATIONSHIPS OF THE PYRENOAMYCETES

by Anders MUNK
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The subject about which I shall have the honour and the pleasure to begin to-day's discussion is presented to us in such a manner that it is open to various interpretations. One might give a historical survey of the taxonomy of the Pyrenomycetes; this would have been a natural interpretation if we were concerned with a well-finished system. This is not the case and as for the history of the problem I will merely refer to LUTTRELL (1951) who gives a very good treatment of this aspect. One might give a list of all morphologic characters in the Pyrenomycetes and a discussion of the possibilities for their application in taxonomy. Or one might consider our subject with a view to its embryological, biological, methodological, or merely logical aspects. But facing the present situation in the taxonomy of the Pyrenomycetes I feel able to introduce the subject in one way only. Since the 7th International Botanical Congress in Stockholm 1950 a number of more or less important papers on taxonomy of Pyrenomycetes have appeared, after a quiet and inactive period of some twenty years. Although great steps have been taken towards the building up of a natural system, there is still so much disagreement between the authors that the situation must be characterized as disharmonic and unsatisfactory. In order to realize this state of affairs it is only necessary to compare the papers of LUTTRELL and MUNK (1953); their titles are essentially the same, but apart from a certain coincidence in names and terms nobody reading them would believe that they treated the same subject. This does not mean that I disregard LUTTRELL, nor that he disregards me; it merely goes to prove that so far nobody has been able to correlate all known data of the case, and that an

embryological basis for a taxonomy (as used by LUTTRELL can at present give another result than a comparative anatomical basis (as used by me). This is the case because neither our embryological knowledge nor our comparative-anatomical knowledge is sufficient to solve the problems at present. On the other hand: it is evident that much new experience and many new suggestions have been published which need a new synthesis. I will try, then, to correlate the European literature unknown to LUTTRELL with his book thus to establish what is at present applicable as bases for the taxonomy of the Pyrenomycetes.

I am not the right man to judge the situation, because I am part of the case myself. On the other hand, my personal concern with the subject may be of some advantage; I shall do my best to present the problems. No doubt my speech will be coloured by my own observations and ideas; but anyone who will confine himself to undebatable statements about this subject will have to confine himself to saying almost nothing. It is my hope that other participants in the discussion will contribute to a balanced and harmonious result of the debate.

And now a brief account of the papers, in alphabetical succession.
LUTTRELL 1951.

This book is based upon a synthetic study of literature concerning the ontogenetic development in different species of Pyrenomycetes. The author himself has previously given good contributions to this literature, but in this book no new observations are published.

I believe that this book will be eagerly read by all pyrenomycologists for many years to come. This illustrates better than many words the lack of such a book, so far. I was amazed to learn from LUTTRELL the number of isolated investigations made in this field of mycology, and how much might be done by compiling their results.

LUTTRELL's emphasis of the bitunicate ascus as a taxonomic criterion seems to me to be the most convincing point in his book. On page 74 LUTTRELL admits certain objections to the division of the Pyrenomycetes upon the basis of ascus structure: the ascostromatic forms *Sphaerognomonia carpinea* and *Ophiobolus graminis* are stated to possess asci of the *Diaportha* type. That statement is correct; but both fungi really belong in *Diaporthales*, and LUTTRELL's emphasis on the ascus structure is even more justified after that. By the way, I should like to mention a fungus which so far has been placed in an ascophymenial group, but the ascus of which is bitunicate: CHADEFAUD (in his interesting studies on ascus-top structures) mentions and figures a « *Nectriacée scolécosporee* » the top-structure of which is like that of *Pleospora* and other fungi with bitunicate asci. It is given as an example (in the opposite way of LUTTRELL's mentioned above) to show that the main lines of the system may be traversed by ascus-structure. The fungus must be *Ophionectria cerea*. I have examined this species and found

it to be a perfectly distinct ascolocular form totally unrelated to *Nectria*. As also emphasized in my paper: the bitunicate ascus is the best and most reliable criterion for placing a fungus among the Ascoloculares — even better than LUTTRELL himself ever realized.

But I should add: the unitunicate ascus is not so good a criterion as the bitunicate ascus. The unitunicate ascus merely shows that a fungus with such an ascus belongs in some other group of Ascomycetes outside the Ascoloculares (and there are many other groups). LUTTRELL's strict bipartition of the Euascomycetes into Unitunicatae and Bitunicatae seems unnatural to me; one should not parallelize a good natural taxon with an evidently heterogeneous group comprising all the rest.

LUTTRELL establishes a series of different types of centrum in the Pyrenomycetes the most important of which are: 1) The *Pleospora*-type: In a stromatic body hyphae grow downwards in dense formation creating a cavity into which the asci grow from below. — 2) The *Nectria*-type. Like the *Pleospora*-centrum except that it is created within a perithecial wall. — 3) The *Xylaria*-type: Asci mixed with paraphyses which spring from the same area as the asci. — 4) The *Diaporthe*-type: Centrum originally solidly pseudoparenchymatous; this tissue disintegrates when the asci are developed; previous records on paraphyses are cited, but considered doubtful. — Later on I shall return to a discussion of these types.

MUELLER et v. ARX 1950.

The paper is an interesting attack on the most difficult of all groups of Pyrenomycetes. As already said, it is rather easy to recognize the ascolocular nature of a fungus by means of the thick-walled, bitunicate ascus; but the subdivision of the group is a difficult problem which has not been fully solved by the authors.

Concerning the main bulk of the group the spore-septation has been used as a primary criterion for the erection of evolutionary series (Entwicklungsreihen), e. g. a *Leptosphaeria*-series, a *Pleospora*-series, a *Didymosphaeria*-series, a *Mycosphaerella*-series (which includes i. a. *Didymella*), and a *Venturia-Gibbera*-series. I consider the delimitation of the *Venturia-Gibbera*-series to be a very good point of the paper; however, I do not fully agree with several of the other groupings for reasons I shall give later on.

The paper is valuable inasmuch as it presents a large amount of new observations and ideas. It suffers from being somewhat fragmentary, though about a hundred species have been examined; even that is not sufficient in this difficult field of mycology.

MUNK 1953.

This paper is based upon my own comparative-anatomical observations on Danish Pyrenomycetes. It has the following disadvantages: my own results are not so well correlated with the literature as they ought to have

been; the system proposed would have been more adequate if the author had been better acquainted with recent literature on the subject. It is deplorable that the author has not been able to employ the new code of nomenclature consistently. Figures are badly wanted; the photographs at the end of the paper are almost without value in the size and reproduction in which they appear.

Quite neutrally, I should like to say that the author has made no attempt to place all fungi examined in families. He has only maintained or erected families which appeared to him to show a characteristic pattern of variation. This is not expressly stated in the paper.

It seems to me that the paper has the following advantages:

It presents a fairly large material of original observations. It is so wide in its delimitation of the subject that probably all important groups are represented; it seems unlikely that large groups are left the later incorporation of which into the system would disturb the picture seriously. It is written in such a way that it may be used a hand-book; in case of a Pyrenomycete presumably belonging in one of the genera treated one may verify quickly and with certainty.

And now we approach the problems of our subject of to-day; we must face the facts: in the numerous cases of disagreement some one or all of these authors must be mistaken. Before we enter into this unpleasant theme, it may be convenient to summarize the points on which we agree:

In Ascohymeniales it is generally accepted that Xylariaceae and Diatrypeae are closely related, and that *Diaporthe* and its relatives constitute a good taxon; further, that Coronophorales is a natural group unrelated to the other groups in question.

In Ascoloculares we agree that *Venturia*, *Coleroa*, *Stigmatella*, etc. constitute a good taxon.

It will be quite impossible for me to cover all important details on this occasion. I shall confine myself to giving one example for the Ascohymeniales and one for the Ascoloculares.

It is very inconvenient that we cannot place Lasiosphaeriaceae among the things on which we agree, because that family seems to me to be the basic group for the main bulk of the ascohymenial Pyrenomycetes. LUTTRELL has placed *Sordaria* with the *Diaporthe*-type of centrum; he has not paid much attention to *Lasio-sphaeria*—curiously enough—because *Lasio-sphaeria* evidently shows another type of development. MILLER (1949) gives a microphotograph of a young *Lasio-sphaeria*-perithecium showing vertical filaments attached above. MILLER interpretes this by placing *Lasio-sphaeria* in Pseudosphaeriales. This is not correct; *Lasio-sphaeria* has a distinct perithecial wall and unitunicate asci and evidently represent the *Nectria*-type of centrum. (I should prefer to call these vertical filaments apical paraphyses; I do not believe that they are homologous to the pseudopara-

phytes in the Pseudosphaeriales as supposed by LUTTRELL. A third type of Pyrenomycetes enters into the picture by LUTTRELL's description of the *Sordaria*-ascus as « at least related to the *Xylaria*-type », and by my emphasis on the similarity between the spores in *Coniochaeta* (close to *Sordaria*) and Xylariaceae. The same tendency towards Xylariaceae is suggested by DANGEARD's statement that in *Sordaria* « some of the cells lining the resulting cavity produce hair-like outgrowths which correspond to paraphyses » (cited from LUTTRELL).

Before drawing conclusions now we must ask: is this family of Lasiosphaeriaceae sensu MUNK a natural family? That implies the most important problem: how can we verify the more or less « natural quality » of a larger taxon? This problem is certainly a central one in this discussion, and I shall take this opportunity to present my opinion of it, because here is a point where we need the result as well as the method.

Helminthosphaeria, *Coniochaeta*, *Sordaria*, *Podospora*, *Lasiosordaria*, *Bombardia* and *Lasiosphaeria* are genera referred by me (and, as I have noticed with considerable relief, by Cl. MOREAU) to the same family: Lasiosphaeriaceae (I know that nomenclatoric considerations will probably result in other names; for reasons of convenience I will use these names, the same as in my paper; they will not cause confusion, I think). If all of these genera appear clear cut throughout, we may say that they are good and undebatable genera — and their relations to one another remain hypothetic, based upon more or less probable inference. But this is not the case here (except for the specialized *Helminthosphaeria* and the homogeneous *Coniochaeta*); the main part of the genera are very difficult to circumscribe satisfactorily. Let us take *Bombardia* as a starting point (type species: *B. fasciculata* Fr.).

From the type species one would define the genus by the cartilagineous plectenchymatic outer wall of the perithecium, by the filiform paraphyses, by the ascus-top containing a refractive sphere, and by the spores: originally cylindric and hyaline appendaged at each end; later on the distal portion of the spore swells and becomes dark-coloured. V. HOEHNEL, who repeatedly occupied himself with *Bombardia fasciculata*, first merged the genus with other fungi showing the same of spore-development (species of *Lasiosordaria*). Later on his discovery of the peculiar perithecial wall made him re-erect the genus for *B. fasciculata*.

So far, I do not know which is best, because these characters occur in different combinations in the family. The spore-character is found in *Lasiosordaria*, accompanied by a perfectly normal perithecial wall.

The cartilaginous perithecial wall is found in e.g. *Podospora appendiculata* and apparently in (Eu-) *Sordaria bombardioides* too. The refractive body is found in *Lasiosordaria* and in some species of *Lasiosphaeria* (in the latter genus apparently concurrent with the presence of appendages on the spores; but highly appendaged spores without a refractive body in

their ascus are common in *Podospora*). And paraphyses are common in the family, but lacking in some Sordariae (this gives no real information, however, because we do not know yet whether they are always apical as in *Lasiosphaeria*; probably they are not). — The primary appendage (better: vestigium) in *Podospora* shows a perfect transition from an almost bombardioid type to a nearly vestigium-less type; and the *Sordaria*-type of spore without appendage but with a gelatinous sheath round the spore does not appear to be essentially different (the germ pores seem to be similar in all species of *Sordaria* and *Podospora*).

As a summary: if we arrange the species according to spore-type we shall get one taxonomy; if we arrange them according to perithecial wall we shall get a different taxonomy; the ascus-structure gives a third taxonomy. This is the very fact to convince us that the whole group is a natural taxon: all species may be described variants of the same set of themes, the variations following the same set of rules. In short: the group can be characterized by what I should like to call « a peculiar and well defined pattern of variation ». This is the point I have been aiming at for the last five minutes: our criterion for the « natural quality » of a taxon is not always and not only the occurrence of certain characters throughout the group. I should like to express this more precisely by saying that the static pattern of a group can be used as an argument in favour of considering the group to be a natural taxon only when it includes several constantly concurrent characters. Our best criterion for such a statement is, in general, the discovery and understanding and description of the pattern of variation in the group (if the group is an unnatural one, the characters which are not constant within the group will not make any pattern at all). — A natural taxon may very well be most strikingly characterized by the fact that one character, which appears to be constant among most other parallel taxa, shows a wide range of variation in the group in question. I admit that the Lasiosphaeriaceae *sensu meo* show variations of several characters which usually are constant in families of Pyrenomycetes: ascus and centrum. The pattern of variation in the ascus top ranges from a structure somewhat similar to that of Xylariaceae (in *Sordaria* and *Coniochaeta*) to one somewhat similar to Diaporthaceae (in *Lasiosphaeria*), and further, comprehends special structures (the refractive sphere); and the centrum shows affinity to Xylariaceae (certain Sordariae and especially *Coniochaeta*), or to Diaporthaceae (especially *Sordaria macrospora* according to my experience), or to *Nectria* (*Lasiosphaeria*). On one condition, this confirms my idea of Lasiosphaeriaceae as the basic group for most Ascohymeniales — and on one condition only: that the centra of the *Xylaria*-type, the *Nectria*-type and the *Diaportha*-type can be demonstrated to be variants of the same basic type and not fundamentally different as supposed by LUTTRELL. And this is more easy to demonstrate than one would think:

If we follow the ontogenetic development, as recorded in the literature, of the three types in question, we find them to be essentially similar till they reach a stage which may be characterized like this: a more or less dense, solid, spheric body of a plectenchymatous or pseudoparenchymatous structure. What happens then in the *Xylaria*- and *Nectria*-type is that the cells in the centre of the body cease to divide themselves forming more pseudoparenchyma and are replaced by outgrowth from more peripheral cell-layers. And I wonder whether such outgrowth of fungus tissue in one direction is not always a hyphal growth? I do not know any examples of filling up a small space with fungus tissue in other ways.

I think we ought to remember that the fungi are hyphal organisms, and that hyphal growth is their natural way of growth. I believe that we shall have to abandon all ideas of a deep morphological understanding of Pyrenomycete-centra by means of investigations in the origin of their paraphyses. — In the *Diaporthe*-type of centrum the central cells in the afore-mentioned body simply keep on dividing themselves; the central tissue maintains its meristematic quality for a longer time than in the other types of centrum. Also here paraphyses occur (I have seen them in nearly all fresh specimens of Diaporthaceae I ever examined; but they are evanescent). They begin to develop when the centrum tissue desintegrates and as they soon dissolve again they may be termed ephemeral. But that does not mean, in my opinion, that they deserve a special term such as « pseudoparaphyses », « metaphyses » or the like. I do not any more lay a great emphasis on paraphyses in morphological considerations in the ascohymenial Pyrenomycetes.

A scheme will show the probable relations between the centrum-types (the *Phyllachora*-type is introduced as a separate type; LUTTRELL included it in the *Xylaria*-type).

The *Xylaria*-type is formally central, characterized by the paraphyses springing from all over the inside of the peridium; their growth is limited by those from the opposite side, and their ends remain free.

In the *Phyllachora*-type the paraphyses are mainly limited to the floor of the perithecium; their growth is limited by the sterile ceiling of the perithecium, and their tips remain free too.

In the *Nectria*-type, the paraphyses mainly grow out from the apical portion of the peridium; their tips meet the hymenium in which a lot of cell divisions and biochemical activity in general take place, and their tips are— quite naturally — obscured by all this tissue.

Coronophorales still seem to represent an unrelated group; so does probably *Calosphaeria*, and other independent groups may have to be added.

If we maintain the rank of order for Coronophorales, I think we shall have to include Lasiosphaeriaceae together with Xylariaceae-Dia-

trypaceae, Phyllachoraceae, Nectriaceae, and Diaporthaceae in one order which covers, in a somewhat emended form, my Sphaeriales.

As an example for the Ascoloculares I will select evolutionary series of *Pleospora* and *Leptosphaeria* and the genus *Didymella* sensu MUELLER & v. ARX (including species which I have wrongly distributed upon several groups in my paper). — Concerning *Pleospora* I shall give a special account of WEHMEYER's monographic papers. — I shall try to show that all these forms constitute one distinct pattern of variation, and the consequence is that they can hardly be distributed upon different evolutionary series (at least no in my opinion).

The spore-types occurring here are: 1°) Muriform and coloured. — 2°) Transversally septate; coloured (or hyaline). — 3°) Bicellular and hyaline. (It is remarkable that most phaeodidymosporous forms belong elsewhere; only *Monascostroma* seems to belong here, as a variant of the phragmosporous type).

The pseudothecium-structure occurs in four types: A) The solid, *Pyrenophora*-like type with thick, sclerotious walls. — B) The plain, subspheric type with a uniform and rather thin wall and without a special periphysoid structure in the papilla. — C) The highly developed type with a thin wall and a periphysoid tissue in the papilla (concurrently with this type of pseudothecium is generally seen a covering of the papilla with dark, spiny hairs, and the spores are often appendaged by what seems to be remnants of a gelatinous sheath). — D) The *Lophiostoma*-type: Papilla laterally flattened, with periphysoids (this type was clearly demonstrated by MUELLER & v. ARX 1950 to be closely related to some *Leptosphaeriae*).

Theoretically we may establish a rectangular, artificial scheme with twelve squares corresponding to the twelve possible combinations of spore- and pseudothecium-characters. Strangely enough every one of these twelve squares is actually occupied by one or more existing fungi. — Shall we, now, follow the spore-lines or the pseudothecium-lines in establishing genera? MUELLER & v. ARX as well as WEHMEYER and others follow the sporeline (till it reaches the *Lophiostoma*-type). The pseudothecium-lines have not been followed, as far as I know (except in *Lophiostoma*), but that is due to the fact that these structures were not yet investigated when the classic genera were established on the basis of spore characters (except, again, the *Lophiostoma*-type; it is recognizable with a hand-lens). I see no reason why the pseudothecium-lines should not equal the spore-lines in importance. I should prefer to have a generic name for each square in the scheme.

WEHMEYER (1946-53).

Other monographic papers on genera of Pyrenomycetes have appeared since 1950 (e.g. MUELLER's works on *Leptosphaeria*, *Pleospora* and *Ophio-*

bolus). But for this occasion WEHMEYER's work requires a special attention because it expressly deals with problems of evolution in the Pyrenomycetes. WEHMEYER has studied a great many collections of *Pleospora*, mainly from North America (where the genus seems to be more difficult than elsewhere) and reports the occurrence of long series of completely intergrading forms between previously described, apparently distinct species. WEHMEYER lays a strong emphasis on the spore-septation; his descriptions and figures of spores are most elaborate, and his terminology proposed to assign the different septa of the spores seems to me to be quite adequate. — The importance of spore-characters in WEHMEYER's opinion is illustrated by the fact that a paper titled « The developmental pattern in the genus *Pleospora* » almost exclusively deals with the spore-characters. WEHMEYER's verification of this importance of spore-characters is not stated in so many words, but it seems to be the fact that it has indeed been possible for him to arrange the spore-types of the genus in a branched system composed of different lines. This may be a good and valid verification, and we learn from this work that the spore-morphology of dictyosporous fungi is worth studying. But the problem is even more intricate. Also the pseudothecium structures as seen in vertical sections show a characteristic developmental pattern in *Pleospora* (see MUELLER's paper on the Swiss Pleosporae), and this pattern has not yet been correlated with WEHMEYER's experience in spore-septation. Further, there is no doubt that other dictyosporous genera certainly interfere with *Pleospora* in the extremely wide sense adopted by WEHMEYER. E.g. *Pleospora Amelanchieris* Wehm. is so close to the very type species of *Cucurbitaria* (*C. Berberidis*) that it can be distinguished from it only by means of a slightly less pronounced septation of the spores (all other qualitative and quantitative characters fit in perfectly well).

In spite of its admirable comprehensiveness, WEHMEYER's work is evidently a torso yet. But it is very probably a good torso, and it gives us a large amount of new information.

I now come to the conclusion of this introduction.

The most important basis for the taxonomy of the Pyrenomycetes is without doubt the separation of all ascolocular forms recognizable by their bitunicate asci from all the rest (which we may call Ascohymeniales; but I believe that is a heterogeneous group which should not be established as a parallel to the Ascoloculares). In other words, from now on we may, consequently, expect a general acceptance of NANNFELDT's central idea.

The rest remains debatable, and I am looking forward with interest to contributions to the discussion. I believe I have dealt with a central point in the Ascohymeniales by my discussion of Lasiosphaeriaceae as the basic group of Sphaeriales *sensu meo*. And I believe to have found a clue to an understanding of the large, central group of Pseudosphaeriales,

as mentioned above. — But I am really unable to say in general which characters should be employed in establishing the better taxonomy which is so badly needed for the Pyrenomycetes. This is due to the fact that a natural group has always developed by going its own ways; it is up to us to trace these ways, and no general rules can be given for this work because the ways are the particular ways of each group. We have to acquaint ourselves thoroughly with each group we want to investigate, and we have to look for a pattern of characteristics, both the static pattern and the pattern of variation. And it is safest to look mainly for the pattern of variation, because the taxa which still remain to be circumscribed are likely to show indistinct static patterns (they would have been found already if their static patterns had been evident).

I hope that my pointing out and definition of the concept « pattern of variation » will be useful for future work.

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DIE SYSTEMATISCHE STELLUNG
DER GATTUNG *PHYSALOSPORA* NIESSL
NACH AUSSCHLUSS
DER GATTUNGSFREMDEN ELEMENTE

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Die Gattung *Physalospora* im bisherigen Sinne ist eine der heterogensten unter den Pyrenomyceten. Die etwa 300 beschriebenen Arten gehören verschiedenen Entwicklungsreihen an und verteilen sich auf mehrere Gattungen.

Ein Original Exemplar der Typusart *P. alpestris* Niessl besteht nicht mehr; von Dr. MUELLER auf verschiedenen alpinen Cyperaceen gesammelte Exemplare sind aber ohne Zweifel mit diesem Pilz identisch. Er gehört zu den Sphaeriales und ist ziemlich primitiv gebaut. Die kleinen, rundlichen, plectenchymatisch aus kleinen, zartwandigen Zellen aufgebauten Perithezien besitzen eine scheitelständige, innen mit fadenförmigen Periphysen besetzte Mündung. Die wenig zahlreichen Asci sind sackförmig und bestehen aus einer einfachen, am Scheitel eine Apikalplatte bildenden, nicht verdickten Membran. Sie sind von schleimig verbundenen, fadenförmigen, oben abgerundeten Paraphysen umgeben. Die verhältnismässig grossen, einzelligen Sporen enthalten ein milchig-körniges oder öliges, schwach bräunliches Plasma.

Nur wenige Arten sind mit diesem Pilz congenerisch wie *P. rhododendri* (Speg.) Rehm oder *P. hyalospora* (Ces.) Sacc. Ferner müssen einige bisher in andern Gattungen untergebrachte Arten zu *Physalospora* gestellt werden, so die Typusarten der monotypischen Gattungen *Pseudophysalospora* v. Höhn., *Acanthorhynchus* Shear, *Trichophysalospora* Lebedeva und *Samarosporella* Linder.

Nach dem Baue der Fruchtschicht beurteilt steht *Physalospora* einerseits gewissen Vertretern der Gattung *Sordaria*, anderseits Gattungen wie *Glomerella*, *Hyponectria*, *Pseudomassaria* und *Griphosphaeria* nahe. *Sordaria brassicae* (Klotzch) Oudem. z. B. unterscheidet sich neben der oberflächlichen Wachstumsweise nur durch die im Alter dunkler gefärbten und mit Anhängseln versehenen Sporen. *Glomerella cingulata* (Stonem.) Schr. et Spauld. anderseits hat bleibend hyaline, durchscheinende, nicht milchig-ölige Ascosporen und ist ferner durch die intraepidermale Wachstumsweise und die *Colletotrichum*-Konidienform ausgezeichnet.

Zahlreiche als *Physalospora* beschriebene Arten sind mit *Glomerella cingulata*, einer äusserst plurivoren Art, identisch. Es sind dies z. B. *P. phormii* Schröter, *P. miyabeana* Fukushi, *P. baccae* Cav. oder *P. cattleyae* Maubl. et Lasnier. Auch *P. tucumanensis* Speg. gehört als eigene Art in die Gattung *Glomerella*.

In anderen Gattungen der Sphaeriales gehören z. B. *P. aurantia* Ellis et Ev., die mit *Polystigma astragali* (Lasch) v. Höhn. identisch ist oder *P. phomatoides* (Mont.) Sacc., das ein klypeales Pseudostroma besitzt und zu *Phyllachora* gehört. *P. bersamae* Syd. hat wie *Phyllachora* gebaute Perithezien, bildet aber Blattflecken und hat keinen Klypeus. Für diesen Pilz besteht die Gattung *Plectosphaera* Theissen, in die noch mehrere bisher bei *Physalospora* untergebrachte Arten gestellt werden müssen.

Zahlreiche bisher als *Physalospora* eingereihte Arten gehören zu den Ascolulares. Ihre Asci besitzen eine doppelte, nach oben meist stark verdickte Membran. Die Fruchtkörper sind parenchymatisch aus derbwandigen Zellen aufgebaut; die Asci wachsen in anfangs geschlossenen, sich bei der Reife durch Wegbröckeln oder Histolyse der Scheitelpartie öffnenden Loculi zwischen oben und unten festsitzenden Paraphysoiden heran. So gebaute « *Physalospora* » - Arten müssen zum grossen Teil mit *Botryosphaeria quercuum* (Schw.) Sacc. vereinigt werden. Dieser meist rindenbewohnende Pilz ist sehr veränderlich und umfasst Formen mit und ohne Stroma. Uniloculare, einen einzigen Loculus enthaltende Formen wurden u. a. als *P. cydoniae* Arn., *P. glandicola* (Schw.) Stev., *P. obtusa* (Schw.) Cke. oder *P. gregaria* Sacc. beschrieben. Die Konidienform gehört in die Gattung *Botryodiplodia*, als solche, sowie als *Sphaeropsis*, *Macrophoma* oder *Diplodia* ist sie in der Literatur unter einigen hundert Namen bekannt. Zu *Botryosphaeria* muss auch der am besten als *P. festucae* (Lib.) Sacc. bekannt Pilz gebracht werden. Für ihn wurde früher die Gattung *Pyreniella* Theiss. begründet. Andere bitunicate *Physalospora*-Arten gehören zu *Guignardia* und *Vestergrenia*.

Summary. The taxonomy of the genus *Physalospora* NIESSL. — *Physalospora* is one of the most heterogenous genera among the Pyrenomycetes. It contains about 300 species. *P. alpestris* Nssl., the type-species of the genus belongs to the *Unitunicatae sensu* LUTTRELL (1951) in the order of the *Sphaeriales*. It is a primitive Ascomycete with relations to genera as *Sordaria*, *Glomerella* and *Hyponectria*. Only 10 of the 300 described species are congeneric with *P. alpestris* and can remain in the genus, all the others must be placed in other genera.

Species as *P. phormii* Schröter, *P. miyabeana* (Fukushi, *P. baccae* Cav. or *P. cattleyae* Maubl. et Lasn. are substrate-forms of *Glomerella cingulata* (Stonem.) Schr. et v. Spauld.; *P. tucumanensis* Speg. is also a *Glomerella*. The species of this genus have *Colletotrichum* conidial-stages.

Other species of *Physalospora* have to be brought to *Polystigma*, *Phyllachora*, *Plectosphaeria* and other genera of the Sphaeriales.

Many species have asci with a double membrane and belong to the Ascoloculares, e. g. *P. cydoniae* Arn., *P. glandicola* (Schw.) Stev. and *P. obtusa* (Schw.) Cke. are unilocular forms of the white-spread and polyphagous species *Botryosphaeria quercuum* (Schw.) Sacc. This fungus contains forms with and without a stroma and the conidial-stage belongs to *Botryodiplodia*. *P. festucae* (Lib.) Sacc. is also a *Botryosphaeria*.

ORGANOGENIE COMPAREE CHEZ LES MELANOSPORA

par Gaston DOGUET

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Nous avons suivi l'évolution des diverses parties du périthèce de plusieurs Pyrénomycètes, depuis le début de l'archicarpe jusqu'à l'évacuation des dernières ascospores mûres. Les détails de nos observations seront publiés dans un mémoire étendu; nous résumons ici quelques-unes de nos conclusions relatives au genre *Melanospora*.

Les *Melanospora Zamiae*, *damnosa*, *ornata*, *Moreaui* (n. sp.), et *parasitica* ont été examinés. La forme de l'archicarpe varie d'une espèce à l'autre, mais dans tous les cas, trois zones, que nous appellerons A, B et C en partant de la base, impossibles à distinguer morphologiquement, donnent naissance à trois parties différentes du périthèce. Chacune d'elles est formée de cellules plurinucléées.

— La zone A émet les filaments recouvrants dont les ramifications viennent envelopper la partie supérieure de l'archicarpe. Ils sont à l'origine de la paroi périthéciale, formée de deux couches, l'externe aux cellules polyédriques, l'interne aux cellules aplaties tangentiellement. La couche interne s'invagine en un point dans l'assise externe, perce à l'extérieur, et forme le col du périthèce ainsi que les périphyses qui tapissent son canal.

— La zone B fournit un plectenchyme nourricier dont les cellules emplissent entièrement l'espace compris à l'intérieur de la paroi (cœur du périthèce). Ce plectenchyme ne pénètre jamais dans le canal du col.

— La zone C est l'ascogone. Elle émet des hyphes ascogènes qui, d'abord plurinucléés en général, passent très vite à l'état binucléé par cloisonnement transversal. L'émission suit de très près la formation du plectenchyme nourricier. Chez les *Melanospora ornata* et *parasitica*, des crochets dangeardiens ont été vus; chez les autres espèces, les asques

naissent par développement direct d'une cellule quelconque de l'hyphe, après fusion de ses deux noyaux.

Les hyphes ascogènes sont réunis en une lame peu épaisse, plane tout d'abord, perpendiculaire à l'axe passant par le col. Elle peut s'étendre jusqu'à la paroi périthéciale, et divise le plectenchyme nourricier en deux parties d'aspect souvent différent, le plectenchyme sus-hyménial et le plectenchyme sous-hyménial, destiné l'un et l'autre à disparaître plus ou moins rapidement après avoir servi de nourriture aux asques.

Plusieurs cas peuvent se présenter :

— Chez les *Melanospora Zamiae* et *damnosa*, la lame hyméniale est tout d'abord sensiblement équatoriale. Elle s'abaisse lors de la croissance des asques qui, finalement, semblent issus d'une zone plus ou moins étendue appliquée contre la paroi périthéciale et à sa base.

— Chez le *Melanospora parasitica*, le plectenchyme sous-hyménial, peu développé, disparaît très tôt. La lame hyméniale, très étendue, vient alors tapisser presque toute la paroi, et les asques croissent de la périphérie vers le centre.

— Chez les *Melanospora ornata* et *Moreaui*, la lame est encore plane, mais les asques ne se développent en abondance qu'au centre. Il se forme alors un nodule central relié à la lame par un pédicelle plus ou moins épais, et l'ensemble présente l'aspect d'un bouton de col. Lorsque le pédicelle s'amincit, les asques partent en rayonnant du nodule central vers la périphérie.

L'organogénie comparée permet donc de réunir dans un même type de développement, d'une part, des périthèces chez lesquels l'hyménium tapisse presque toute la paroi, les asques croissant de la périphérie vers le centre, d'autre part, des périthèces chez lesquels l'hyménium se condense en un nodule central, les asques croissant du centre vers la périphérie.

Au terme de cette évolution, nous avons rencontré le *Thielavia setosa*, forme à périthèces toujours clos, qui, par ailleurs, présente de nombreux caractères de *Melanospora* : asques et ascospores identiques, biologie semblable (obligation de cultures mixtes avec des espèces fongiques convenables pour obtenir des périthèces). L'organogénie montre que, chez cette espèce, les asques partent d'un nodule central vers la périphérie. Il est très probable que l'un des passages de la forme close à la forme ouverte du périthèce des Pyrénomycètes se fait au niveau des genres *Melanospora* et *Thielavia*.

AN ANATOMIC STUDY IN THE GENUS *NECTRIA* FR.
WITH REMARKS ON THE PATTERN
OF VARIATION WITHIN THE GENUS

by Anders MUNK

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To-day's taxonomy of the genus *Nectria* Fr. is mainly based upon the conidial stages, in accordance with WOLLENWEBER (1926). This is, in fact, an unsatisfactory state of affairs: first, modern mycologists generally base taxonomy of Ascomycetes on studies in the ascigerous stage; secondly, the ascigerous stages of *Nectria* spp. do show interesting aspects which seem to be valuable in taxonomy.

I have examined some 14 species of the genus. Among these I shall exclude *Nectria Peziza* from the genus; and *N. ochroleuca* (a tropical species) does not seem to be closely related to the North European species; consequently, this paper mainly deals with 12 species of *Nectria*.

I have studied these 12 species in sections and squash mounts made from herbarium material of mature perithecia; I should like to mention the following results of the investigation, including a revised diagnosis of the genus and some remarks on its pattern of variation.

Nectria Fr. 1849 (including *Scoleconectria* Seaver 1909, p. 197).

Type species proposed: *Sphaeria cinnabarina* Tode ex Fr. (instead of *Sphaeria Peziza* Tode ex Fr. proposed by SEAVER, 1909).

Basal *stroma* (if present) whitish to reddish, composed of vertically seriate cells. — *Perithecia* generally some shade of red or orange, turning violet with KOH, solitary or gregarious, entire or collapsing when dry, $\pm$ distinctly papillate. — *Peridium* $\pm$ cartilagineous, the outer cells in the free portion of the perithecia thick-walled to very thick-walled (almost without lumina); the inner cell-layers of the peridium generally composed of flattened cells; but in most species it is difficult to establish a clear difference between two fundamentally different layers. — *Ostiolum* periphysate, often showing a periphysis-like structure of the surface as well as inside; this structure delimits an *ostiolar area* of *external periphyses* round the porus (evidently an eversion has taken place of the originally inner layers forming the ostiolum). Internal periphyses generally springing from a hyaline cushion of thinwalled, isodiametric cells below the porus. The porus is very narrow at the top, but wider downwards, so that the peridium tapers into an *ostiolar* edge towards the porus. — *Centrum*: Asci springing from most of the inside of the perithecium, clavate-cylindric, thin-walled, generally without a distinct apical apparatus. Paraphyses (at least the most

conspicuous ones in sections) springing from a ring-shaped area beneath the periphysisbearing cushion, in the mature perithecium bending downwards at a rather sharp angle and thus mixing with the asci. These *apical paraphyses* are generally 1-5 μ thick, articulated, and branching downwards at acute angles. — *Spores* typically 2-celled, oval to fusiform, slightly or not constricted, in most species hyaline and smooth (a few species have $\pm$ brownish and rough spores; and one species has long, worm-like, pluriseptate spores). In one group of the genus the spores gemmate in the ascus, forming numerous allantoid microconidia.

Taxonomy. The confusion in the taxonomy of *Nectria* (most strikingly demonstrated by WEESE in his numerous excellent papers on Nectriaceae seems to be due to the fact that ascus and spore characters do not help very much to an understanding of the pattern of variation in the genus (except the — largely overlooked! — roughness of the spores in certain fungicolous species). As repeatedly pointed out by v. HOEHNEL and by WEESE the investigation of the perithecium-structure as seen in (exactly!) median sections of perithecia is necessary for the exact description and safe determination of a *Nectria*; The authors mentioned (especially WEESE) have given good contributions to the solutions of problems in *Nectria*; but even they do not seem to have had a clear conception of the pattern of variation.

Only thirteen species are examined by me; and many other species may contribute to a much better understanding. Nevertheless I believe to have traced certain important features of the pattern of variation in *Nectria* — as follows:

The species examined distribute themselves upon three clear cut groups (except the tropical species *N. ochroleuca* (Schw.) Berk.).

1) Generally stromatic species with relatively large perithecia which do not collapse when dry; a distinct ostiolar area of cylindric cells is present. Parasites or saprophytes of primary incidence on woody plants. (Including *N. coccinea*, *N. cucurbitula*, *N. cinnabarina*, *N. galligena*, and *N. punicea*).

2) $\pm$ Stromatic species with vertically collapsing (cup-shaped) perithecia without a differentiated ostiolar area. Allantoid microconidia formed in the asci by gemmation from the poorly, in one species abnormally, developed spores. On woody plants; probably saprophytes of primary incidence. (Including *N. Coryli*, *N. subtersa*, and *N. cylindrospora*).

3) (Generally) slightly stromatic or non-stromatic species with $\pm$ ovate perithecia; peridium occasionally thin (laterally collapsing); a strongly developed ostiolar area of clavate cells is present. Spores smooth and hyaline to rough and brownish. Growing on old stromata or fruit-bodies of other fungi, or on wood or bark as saprophytes of secondary or higher incidence. (Including *N. sanguinea*, *N. Magnusiana*, *N. modesta* and *N. cosmario-spora*).

The collapsing (or not) of perithecia appears to be correlated with important morphological structures and to be an elementarily physical consequence of these. Perithecia in which the weakest point (the thin ostiolar edge delimiting the porus) is reinforced by a palisade of external periphyses do not collapse as to become cup-shaped (They may collapse laterally in case of a thin peridium of the body). If the palisade is absent, the perithecium will collapse to cup-shape when dry.

It is evident that this study in a small number of North European species of this large and mainly tropical genus should not be interpreted as a serious attempt to a new taxonomy of *Nectria*. It has been my intention merely to initiate a revision of the genus on the basis of studies in the ascigerous stage.

DES DISCOMYCETES BITUNIQUES

par Emil MUELLER

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Un des faits les plus importants de la nouvelle taxonomie des Ascomycètes est l'introduction de groupes systématiques basés sur la structure des asques. Il résulte de cette transformation dans la systématique des Ascomycètes de nombreuses imprécisions, obscurités ou erreurs. Nous accueillons avec d'autant plus de reconnaissance les recherches qui essayent de tirer les grandes lignes d'ensemble et les traits généraux. Deux auteurs surtout ont rassemblé en grand nombre les résultats — en partie contradictoires — de différents savants, les ont ordonnés en un système précis, sans toutefois entrer dans les détails.

NANNFELDT (1932) distingue, d'après la formation des asques dans les fructifications, trois groupes : les Plectascales, les Ascohyméniales et les Ascoloculares. Ce système, dans l'essentiel repris par GAEUMANN (1949), considère en premier lieu les fructifications dans leur ensemble, mais sans négliger la structure détaillée des asques parmi les groupes différents.

LUTTRELL (1951) fait un pas de plus en ne caractérisant ses groupes que d'après la structure de l'asque. Il distingue d'une part les Unituniqués, qui correspondent plus ou moins aux Ascohyméniales et Plectascales de NANNFELDT, caractérisés par des asques à parois délicates, souvent pourvus d'appareils apicaux, et d'autre part les Bituniqués, équivalant aux Ascoloculares au sens de NANNFELDT et dont les asques comprennent deux membranes.

Les deux auteurs sont d'accord sur le fait que les Discomycètes pourraient être divisés en plusieurs ordres nettement distincts, mais n'en

appartiennent pas moins aux Ascohyméniales, respectivement aux Unituniqués. Ces derniers temps j'ai étudié quelques champignons des lichens appartenant aux Lécánorales (sens de NANNFELDT). Ils frappent par l'épaisseur des parois ascales, et au premier abord pourraient être pris pour des Bituniqués. Les formes comme *Lecanora subfusca* (L.) Arch., le type de *Lecanora* Arch., ont des asques claviformes, à paroi épaisse. Contrairement aux formes bituniquées que je connais, les asques présentent une intensive réaction à l'iode, ils ne se détachent que difficilement de l'hyménium, et possèdent d'après ZIEGENSPECK (1926) un apex d'isolichénine. Selon NANNFELDT (1932) la paroi ascale ne comprend ici qu'une seule couche, ce qui justifie leur place parmi les Ascohyméniales, resp. les Unituniqués.

Sur la base de ces faits il n'y aurait pas de raison de revenir sur la question. Mais, en fait, il se trouve des Ascomycètes qui morphologiquement appartiennent aux Discomycètes, et à cause de l'épaisseur des parois ascales font partie des Lécánorales, mais s'en distinguent pourtant totalement. C'est le cas de *Patellaria atrata* Fr., décrite et dessinée par exemple par NANNFELDT (1932).

Le champignon forme des apothécies allongées, d'environ 1 mm. de long, largeur: 300-600 μ , hauteur. 200-300 μ , enfoncées dans le substrat à la base. Il se développe un excipulum assez important, jusqu'à 80 μ de profondeur. Celui-ci se compose vers la base de cellules brunes et gobeuleuses, à parois épaisses, qui s'allongent vers le haut et, sans limite distincte, font place à l'hypothèque plus claire. Sur les côtés l'excipulum s'amincit; les cellules allongées et hyalines qui le composent sont arrangées en files parallèles, dirigées perpendiculairement à la surface; ce n'est que vers l'extérieur qu'elles brunissent jusqu'à devenir opaques. Les asques sont claviformes à cylindriques, ont une solide paroi comprenant deux membranes. Ils mesurent 100-150 $\times$ 15-20 μ , sont largement arrondis au sommet, à la base nettement pédiculés, octosporés. Ils sont entourés de paraphyses filiformes, hyalines, mais qui s'élargissent et se colorent en brun vers la pointe. Les spores sont claviformes, hyalines, avec 5-8 parois transversales; elles mesurent 34-44 $\times$ 7-10 μ .

Les asques de *Patellaria atrata* se distinguent surtout de ceux de *Lecanora subfusca* par la réaction de l'iode négative et avant tout par la manière d'éjaculer les spores. On peut en effet examiner que d'abord la membrane extérieure éclate, l'intérieure poussée par la pression s'avance aussitôt, et les spores sont libérées l'une après l'autre par l'opercule. *Baggea pachyasca* Auersw. et *Scutula pandani* E. Müll. ont un comportement identique. Il est donc évident que la paroi ascale des champignons nommés se compose de deux membranes, et ne s'écarte en rien du type des Bituniqués, comme nous le savons par exemple pour *Leptosphaeria*.

CHADEFAUD (1942) a remarqué exactement le même comportement de la paroi ascale chez un champignon qu'il nomma *Pezicula* sp., mais qui

pourrait tout aussi bien être une *Patellaria*. En décrivant une nouvelle espèce (*Patellaria Finkii*), PETRAK (1923) a souligné que sous maints aspects le champignon rappelait le type « myriangial »; la notion « myriangial » devant être prise comme équivalente dans ce cas-là à « ascoloculaire ».

Sans me laisser aller à des spéculations prématurées, vu le petit nombre de formes étudiées, il me semble possible de comparer des champignons tels que *Patellaria atrata* avec les Dothioracées. Toutefois, la structure des fructifications est totalement différente, ce qui parle contre une relation directe avec les Dothioracées. Par contre, on trouve chez les formes ayant des spores semblables ainsi que chez *Baggea* la tendance à une augmentation des spores formées dans l'asque (cf. *Hariota* Karsten, *Sydowia* Bres.). On se rapproche alors de la supposition que les Dothioracées d'une part et les champignons du type de *Patellaria atrata* d'autre part pourraient être déduits d'une forme primitive commune. On ne peut pour l'instant déterminer si *Patellaria* appartient également aux Dothiorales sensu MUELLER et VON ARX (1950) ou représente une autre tendance de l'évolution.

2. — CLASSIFICATION ET PHYLOGENIE DES GASTEROMYCETES

RAPPORT

CLASSIFICATION ET PHYLOGENIE DES GASTEROMYCETES

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La position phylogénétique des *Gastéromycètes* est un sujet fortement contesté sur lequel des opinions tout à fait contradictoires ont été présentées et sont encore présentées. Ce rapport donnera un aperçu des diverses théories et des principales raisons, apportées *pro et contra*.

Schématiquement on pourrait dire qu'on a cherché l'origine des *Gastéromycètes* à trois endroits, à savoir parmi les *Aphyllaphorales*, parmi les *Agaricales* et parmi les *Tubérales*, et nous allons examiner ces trois hypothèses l'une après l'autre, commençant par les *Aphyllaphorales*.

Que les *Gastéromycètes* proviennent des Hyménomycètes primitifs ressemblant aux *Corticaceae* est une opinion fort répandue; à cet avis se rallie par exemple E. FISCHER dans son traité bien connu sur les *Gastéromycètes* dans les *Natürliche Pflanzenfamilien* (1933) et cette hypothèse revient dans plusieurs manuels récents (GREIS, 1943; GÄUMANN, 1949). Ainsi GREIS écrit: « von den *Thelephoraceae*, insbesondere von den *Corticiae*, dürften sich die *Gastromycetes* herleiten. Die flächenförmigen Fruchtkörper der *Thelephoraceae* bieten alle Entwicklungsmöglichkeiten in sich und es lassen sich aus ihnen nicht nur konsolen- und hutartige, sondern auch die knollenförmigen Fruchtkörpern ableiten » (*op. cit.*, p. 309).

Cependant, cette dérivation est, comme le signale GÄUMANN, tout à fait hypothétique, et des formes intermédiaires, supportant la théorie, ne sont pas connues. A notre connaissance c'est LOHWAG (1924) — y adhérant lui aussi — qui a le mieux systématisé les arguments favorables et nous les citons ici, ce sont :

1°) La disposition irrégulière des basides chez les *Plectobasidiineae*, ce que LOHWAG retrouve parmi les *Hypochnaceae*. 2°) Le nombre sporal varie, parfois dépassant 4, caractère que LOHWAG interprète comme primitif et qui a été rencontré aussi parmi les *Hypochnaceae*. 3°) Les stérigmates sont insérés un peu latéralement chez certains *Gastéromycètes* (*Tulostoma*), disposition également trouvée parmi les *Hypochnaceae*. 4°) Le

capillitium de plusieurs Gastéromycètes, considéré par LOHWAG comme homologue des spinules des *Hymenochaete* et de structures analogues chez divers *Thelephoraceae*.

Quelle que soit la valeur de ces caractères, il nous semble qu'on peut sans difficulté les interpréter comme de pures analogies.

Enfin, FISCHER a présenté un argument cytologique en signalant que — autant qu'on le sache — les Gastéromycètes correspondent aux *Hypochnaceae* dans leur alternance de phases, c'est-à-dire que les spores sont binucléées; par conséquent, le mycélium appartient en entier à la dicaryophase, aucun mycélium primaire et monocaryotique ne se développant. Sans prendre position sur la valeur de cet argument, il est à remarquer que, au moins par exception, le mycélium monocaryotique peut atteindre une étendue considérable chez les Gastéromycètes; ainsi on a synthétisé des mycorhizes avec un mycélium monocaryotique de *Scleroderma aurantium* (N. FRIES, 1942).

Cependant, il faut observer que les partisans de cette théorie eux aussi ont souligné l'étroite affinité entre certains Gastéromycètes et certaines Agaricales, même s'ils n'en tirent pas toutes les conséquences possibles. Par là nous abordons le problème qui a été au centre des discussions pendant ces dernières années, à savoir les relations entre les Gastéromycètes et les Agaricales. Après de recherches nombreuses faites par BUCHOLTZ, LOHWAG, et d'autres, et plus récemment encore, grâce à toute une série de travaux remarquables de l'école française, plusieurs cas d'une affinité incontestable entre les Gastéromycètes et les Agaricales ont été révélés, surtout en raison de ressemblances dans les caractères sporaux.

Ainsi on a signalé une connexion entre les *Russulaceae* et les *Asterosporaceae* (cf. MALENÇON, 1931), entre les *Rhodophyllus* et les *Richoniella* (ROMAGNESI, 1933), entre les *Boletaceae* et les *Rhizopogon* (cf. R. HEIM, 1934) et entre différents groupes de *Secotium* (genre selon R. HEIM fortement hétérogène) et d'un côté les *Conocybe* et les *Bolbitius*, de l'autre des *Cortinarius*. Il semble superflu de donner un compte rendu détaillé de tous les faits indiquant cette affinité, puisqu'elle n'est contestée par personne, chose digne d'être soulignée.

Cependant, cette interprétation nous amène à des conséquences fort importantes pour le système des Gastéromycètes. Elle nous force à admettre plusieurs séries d'évolution, indépendantes les unes des autres et sans correspondance avec les coupures admises dans le système ancien. Ainsi, le groupe des *Asterosporaceae* d'après MALENÇON (1931) puis DODGE et ZELLER (1937) est composé de représentants de cinq familles différentes dans le système de FISCHER, à savoir les *Hymenogastraceae* (*Gymnomycetes*, *Martellia*, *Octaviania*, *Sclerogaster*), les *Hysterangiaceae* (*Clathrogaster*, *Maccagnia*), les *Hydnangiaceae* (*Arcangeliella*, *Hydnangium*), les *Sclerodermataceae* (*Lycogalopsis*) et les *Secotiaceae* (*Elasmomyces*, *Macomantes*).

Si l'opinion est unanime — on en est même arrivé à la certitude — qu'il existe plusieurs séries d'évolution, où nous avons à une extrémité des formes agaricoïdes, à l'autre des formes gastéroïdes, les avis sont très différents quant à la direction de cette évolution, autrement dit à savoir si les Gastéromycètes doivent être considérés comme des ancêtres des Agaricales ou *vice versa*. Ces dernières années, ce problème a été l'objet d'une discussion fort intéressante, surtout entre Roger HEIM et SINGER, et dans les lignes suivantes nous allons faire un bref exposé des arguments, présentés *pro et contra*, et principalement tirés des ouvrages des auteurs mentionnés (HEIM, 1948, 1951; SINGER 1951 a, b).

Les arguments sont très variés, d'ordre morphologique, tératologique, cytologique, écologique et phytogéographique et nous aborderons d'abord les faits morphologiques.

L'homologie entre le péricidium des Gastéromycètes et les voiles des Agaricales est indubitable et elle a joué un rôle important, invoquée des deux côtés dans les discussions phylogénétiques. Du point de vue téléologique il est évidemment plus facile d'interpréter les voiles des Agarics comme des traces d'un péricidium; il serait moins compréhensible que ces structures se soient développées pour ainsi dire à l'avance, avant que le stade gastéroïde soit atteint. D'autre part, on le sait, les voiles chez un grand nombre d'Agaricales ont une origine secondaire, les carpophores étant au début gymnocarpes; il s'agit donc de « pseudoangiocarpie ». En outre, il semble correspondre à une opinion générale parmi les agaricologues que les Agarics les plus voilés, notamment les Amanites, sont fort différenciés, comme l'indiquent aussi certaines données cytologiques (KUHNER, 1945). KUHNER signale, en effet, que « les voiles se rencontrent surtout chez les espèces relativement différenciées par ailleurs ».

Les caractères sporaux nous donnent des critères fort importants. Comme on le sait, les spores des Agaricales sont en général asymétriques, ce qui se rapporte à leur mécanisme actif de libération, tandis que celles des Gastéromycètes sont symétriques, la libération des spores étant passive. Comme l'a surtout souligné HEIM, il y a, cependant, plusieurs Gastéromycètes, possédant des spores nettement asymétriques, et, chose fort intéressante, précisément chez ceux les plus étroitement apparentés aux Agaricales.

Envisageons, par exemple, la série des *Russulaceae-Asterosporaceae*, et nous trouverons des spores asymétriques aussi chez plusieurs représentants gastéroïdes. MALENÇON nous dit (1931, p. 385): « *Mac-Owanites* montre des spores d'une forme absolument semblable à celle des Russules; elles sont d'une asymétrie accentuée et fortement redressée vers l'axe de la baside. Tout en s'amointrissant, ces caractères se retrouvent dans *Elasmo-mycetes* et *Gymnomyces*, mais chez *Arcangeliella* on n'a plus de redressement, la spore est simplement asymétrique, la face ventrale très renflée.

Une disposition analogue, quoique beaucoup plus faible, se note encore dans un *Hydnangium* — *H. Ravenelii* Bk. — et fait passer aux autres espèces du genre — *H. galatheium* Q., *H. carneum* Wallr. — et à *Martellia*, dont les spores sont simplement ovoïdes, la face dorsale et la face ventrale s'étant ici accrues d'une égale manière comme aux premiers âges de la spore. Avec *Clathrogaster*, *Octaviana*, on a des spores constamment ovoïdes et tendant même dans bien des cas à devenir subglobuleuses. Enfin, chez *Sclerogaster*, la forme sphérique est la règle ».

Il semble incontestablement plus facile de voir dans cette asymétrie sporale une survivance d'un stade agaricoïde que de la considérer comme une forme antérieure à celui-ci. Cependant, il faut signaler les spores presque symétriques, orthotropes, de certaines espèces de la section *Pelliculariae*, c'est-à-dire des Russules les plus proches des *Macowanites*. On est naturellement tenté de faire l'objection analogue qu'il est plus facile de voir à ce sujet un phénomène de survivance qu'un début de réduction.

A propos du nombre sporal, se manifeste aussi un certain intérêt. Les Gastéromycètes ont, en général, des basides tétraspores, mais il y a des exceptions assez nombreuses. Retournons aux *Asterosporaceae* et nous citerons encore une fois MALENÇON (*op. c.*, p. 383) : « Les basides sont également atteintes par la régression générale. Constamment tétraspores dans *Lactarius* et *Russula*, elles le sont encore chez *Elasmomyces*. Tantôt tétraspores dans *Arcangeliella* et *Martellia*, parfois trispores mais plus régulièrement bispores dans *Mac-Owanites*, elles ont toujours deux stérigmates chez *Gymnomyces*, *Clathrogaster*, et un seul dans *Hydnangium* (*H. Stephensii*, *galatheium*) », etc...

Donc, il s'agit certainement d'une réduction du nombre sporal, ce qui, selon MALENÇON et HEIM, révèle aussi que les formes gastéroïdes seraient des formes dégradées. D'un autre côté, il y a aussi des Gastéromycètes octospores, comme *Tulostoma*, preuve de primitivité selon LOHWAG.

On a aussi fait attention aux phénomènes tératologiques. Il est bien connu que parmi certains Agarics, par exception, se rencontrent des formes anormales, aux traits nettement gastéroïdes, traduisant sans doute une bonne preuve de l'affinité des Agaricales avec les Gastéromycètes. Un nouveau cas en est récemment signalé par SINGER (1951 b), à savoir certains *Cortinarii* de la Terre de Feu, parmi lesquels il a trouvé de nombreux spécimens ressemblant aux *Thaxteromyces*, genre gastéroïde, proche des *Secotium* et existant au même endroit. C'est donc un fait indubitable, mais interprété tout à fait différemment par SINGER et par HEIM. Celui-ci les regarde comme des étapes de dégradation des Agaricales vers les Gastéromycètes, celui-là, au contraire, voit dans ces formes gastéroïdes un phénomène atavique, suite de leur descendance des Gastéromycètes.

Des arguments cytologiques ont aussi été présentés, surtout par KUHNER nous rappelant que le manque de boucles doit être considéré comme une

condition secondaire, avis que font valoir HEIM et SINGER. Nos connaissances relatives à la présence ou à l'absence de boucles sont assurément assez fragmentaires, mais, à ce qu'il paraît, des formes sans boucles sont beaucoup plus fréquentes chez les Agaricales que chez les Gastéromycètes. Il est en tout cas intéressant que les *Russulaceae*, du moins en général, manquent de boucles, tandis que leurs supposés parents parmi les Gastéromycètes sont bouclés, ce qui, en soi, porte à croire à la primitivité de ceux-ci. Des recherches ultérieures sur ce point nous semblent très désirables.

On a aussi invoqué des arguments écologiques. Plusieurs auteurs considèrent la vie hypogée, si fréquente chez les Gastéromycètes, comme un phénomène de dégradation, équivalent, en quelque sorte, à l'adaptation xérophytique de la plupart des Gastéromycètes « secs » (HEIM, 1951, p. 139).

Certains faits phytogéographiques sont d'un intérêt indubitable pour ce problème. CUNNINGHAM (1944) a présenté quelques données statistiques quant aux Gastéromycètes d'Australie et de Nouvelle-Zélande; le nombre total des espèces y est 183, dont 103 ou 56 % sont endémiques, inconnues dans d'autres parties du monde. Il faut ajouter que CUNNINGHAM ne compte qu'environ 450 espèces de Gastéromycètes dans le monde entier. En somme, les Gastéromycètes ont une distribution fort partagée, comprenant un grand nombre de formes endémiques, surtout dans des régions connues pour une flore ancienne, comme l'Australie, la Nouvelle-Zélande, l'Afrique du Sud, ce qui semble indiquer un grand âge chez les Gastéromycètes.

Après cet exposé succinct d'arguments principaux, invoqués dans la discussion phylogénétique, nous ajouterons quelques remarques plus générales. Si l'on suppose que les Gastéromycètes sont primitifs, on est forcé d'admettre les Agaricales comme une coupure polyphylétique, composée de sommets de plusieurs séries de développements, ce qui implique nécessairement que l'asymétrie sporale et le mécanisme fort particulier de libération des spores se sont développés plusieurs fois indépendamment et en détail de la même manière. Ultérieurement, cette hypothèse comporte que les Agaricales — au moins certains d'entre eux — et les Aphyllophorales ont des origines tout à fait différentes, à moins qu'on ne considère pas les Aphyllophorales comme des Agaricales dégradées. Telles sont pour le système des Basidiomycètes les conséquences de cette théorie sur la primitivité des Gastérales, ce qui est, peut-être, sa charge la plus grave.

Or, d'un autre côté, les Gastéromycètes constituent un groupe, pauvre en espèces mais pourtant montrant une diversité étonnante de types différents d'organisation, entre autres des familles sans doute fort différenciées comme les *Nidulariaceae* et les *Clathraceae* qui dérivent évidemment des *Hymenogastreae*. Il semble très difficile de se figurer une évolution des Agarics jusqu'aux Gastérales hypogées, c'est-à-dire une forte dégradation, et ensuite de là une évolution jusqu'aux champignons les plus différenciés qui existent. D'ailleurs il est notoire que des coupures anciennes sont peu riches

en espèces mais pourtant variées, tandis que les groupes les plus récents montrent une abondance en espèces monotones. On pourrait mettre en parallèle les séries des *Araliaceae-Umbelliferae* et des *Capparidaceae-Cruciferae*. Pour les mêmes raisons, les Agarics, groupe nombreux et uniforme, devraient être plus jeunes que les Gastéromycètes.

Comme le démontre ce bref examen, il y a des raisons excellentes qui appuient ces deux théories et — chose plus frappante — elles luttent toutes les deux avec des difficultés importantes. Cependant, l'opinion voyant en des Gastéromycètes des Agaricales dégradées a un avantage incontestable sur l'opinion opposée, car elle explique en effet l'origine des Gastéromycètes. Si, par contre, l'on regarde ceux-ci comme les ancêtres des Agaricales, il reste encore à résoudre le problème de l'origine des Gastéromycètes. Il y faudrait encore une hypothèse, ce qui, en soi, est indubitablement un inconvénient. En général, les partisans de cette hypothèse ont cherché cette origine parmi les Aphyllophorales et l'on revient à la doctrine que nous avons citée en guise d'introduction, et qui est la base de leur argumentation.

Cependant, il y a encore une hypothèse sur ce sujet que nous allons finalement présenter, à savoir que les Gastéromycètes trouvent leur origine parmi les Tubérales. Cette idée se laisse entrevoir déjà chez BESSEY (1935), mais elle a surtout gagné en intérêt grâce aux recherches cytologiques de GREIS sur *Tuber aestivum* et *T. brumale* (1945). GREIS fait part de la découverte extrêmement intéressante de boucles chez ces Ascomycètes et il conclut (*op. cit.*, p. 630) : « Wenn man dann noch die Fruchtkörperorganisation der hypogäischen Basidiomyzeten (*Hymenogastraceae*, *Hysterangiaceae*) in Vergleich zieht, so zeigen sich derartige Uebereinstimmungen morphologischer, entwicklungsgeschichtlicher und physiologischer Tatsachen, dass es nicht zuviel gesagt erscheint, wenn man einen Teil der Gastromyzeten von tuberaceenähnlichen Ascomyzeten herleitet ».

Cette pensée de GREIS est incontestablement originale et s'appuie sur de bonnes raisons. Tout d'abord les Gastéromycètes hypogés et les Tubérales présentent une conformité très étendue en caractères morphologiques, histologiques et microchimiques, ainsi que l'observaient notamment BUCHOLTZ et FISCHER, interprétant ce fait, cependant, comme un exemple très élégant de convergence, donc une analogie et non une homologie (cf. HOLM, 1949). Comme l'a montré GREIS, il y a aussi une ressemblance cytologique remarquable. Il signale chez ces deux espèces de *Tuber* un mycélium dicaryotique bien développé, chose très remarquable vue à la lumière de l'étude splendide de MARTENS (1946) sur la sexualité des Ascomycètes. D'après MARTENS, il est évident que la doctrine claussénienne généralement acceptée, selon laquelle tous les Ascomycètes possèdent une dicaryophase comme les Basidiomycètes, est erronée et fort exagérée. En effet, ces espèces de *Tuber* sont presque les seuls Ascomycètes chez qui

l'existence d'une dicaryophase, comparable à celle des Basidiomycètes, est démontrée d'une manière convaincante. Il est évidemment fort probable que les Basidiomycètes ont leur origine parmi des Ascomycètes possédant une dicaryophase bien développée; chez les *Tuber*, en plus, les crochets se sont transformés en boucles.

Ici s'ouvrent des perspectives vers la phylogénie des Basidiomycètes en général, question qui dépasse notre sujet et que nous n'aborderons pas. Nous nous bornons à envisager une *Tuberaceae* imaginaire, pourvue de dicaryophase et de boucles, et dont les asques se transforment en basides. Comment la distinguer d'un Gastéromycète hypogé? Une telle transformation des asques en basides n'a certainement rien d'impossible ou d'absurde — au contraire, de toute nécessité, elle doit s'être produite une fois, quelle que soit l'origine des Basidiomycètes.

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LA CLASSIFICATION ONTOGENIQUE DES GASTROMYCETALES

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A la classification purement physionomique des Gastromycétales en 4 groupes (Hyménogastrées, Lycoperdées, Nidulariées, Phalloïdées) et à la classification morphologique de FISCHER (1933) qui, se fondant sur le plan structural de la gléba, distingue 4 modes (lacunaire, coralloïde, mehrhütig et einhütig), nous proposons de substituer une classification ontogénique, qui tient compte en outre de l'histoire du développement de la gléba. 5 types s'observent.

Dans le type réticulé, des surfaces basidifères qui rappellent des lames d'Agaric s'anastomosent en un réseau. Le développement est auléiforme (de *auléum*, rideau de théâtre) chez les Hyménogastracées, où des bourrelets pendent au plafond d'une cavité périidiale comme les lames d'Agaric dans la chambre hyméniale du carpophore. Il est coralloïde quand les bourrelets s'élèvent de la base du périidium comme des branches de corail à partir d'un tronc, atteignant la paroi périidiale sans se souder à elle : Hystérangiacées, Clathracées, Phallacées.

Dans le type schizogène, les chambres, que tapisse encore un hyménium, naissent par écartement mécanique des hyphes qui forment leurs parois : Rhizopogonacées, Lycoperdacées, Géastracées, Nidulariacées.

Les loges de la gléba apparaissent par voie lysogène dans la petite famille des Leucogastrées.

Ailleurs, la gléba ne se creuse pas de chambres, ne possède pas de surfaces hyméniales; elle présente des îlots d'hyphes lâches et basidifères, séparés par des veines stériles; c'est le type insulaire (*insula*, île): Sclérodermatacées, Pisolithacées, Astréacées, Calostomatacées, Sphaerobolacées.

Enfin, un type uniforme est celui des Gastromycétales dont la gléba n'est pas creusée de chambres, ne comporte pas d'îlots basidifères; les basides sont éparses dans toute son étendue: Glischrodermatacées, Tulostomatacées.

Cette classification ontogénique nous paraît propre à suggérer des rapprochements entre Gastromycétales possédant une même histoire de leur gléba.

3. — *HYPHOMYCETES AQUATIQUES*

RAPPORT

AQUATIC HYPHOMYCETES

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Until recently most students of freshwater aquatic fungi have confined their attention to Phycomycetes. However, in the last twelve years it has been recognized that other groups of fungi play an important part in the decay of submerged organic material. There is a rich flora of Ascomycetes (both Pyrenomycetes and Discomycetes) growing under water on submerged dead stalks of reed-swamp plants in lakes, and many aquatic members of Sphaeropsidales grow in the same habitat. However, we are now concerned with the aquatic Hyphomycetes (Moniliales) that grow especially on the leaves of broad-leaved trees and shrubs decaying in ponds, lakes and, above all, in quickly-flowing streams. It is to be noted that there are, apparently, no submerged aquatic Basidiomycetes.

The aquatic Hyphomycetes with which I have been mainly concerned (INGOLD 1942, 1943a, 1943b, 1944) occur abundantly on submerged decaying leaves of such trees as oak (*Quercus*), alder (*Alnus*) and willow (*Salix*). Leaves that are becoming skeletonized bear particularly rich crops of these fungi. If such leaves are brought into the laboratory and a day later are examined submerged in a petri dish of water under the low power of the microscope, these Hyphomycetes are easily seen. Often on a single leaf several species are present. It seems almost certain that these fungi are largely responsible for the decay of these leaves. The branched septate mycelium occurs in the tissues of the leaf, especially in the petiole and larger veins, and the conidiophores bearing the conidia project into the water. The spores are produced, liberated and normally dispersed below water.

A few of these fungi had already been described by de WILDEMAN (1893, 1894, 1895), but he did not recognize them as forming a characteristic flora. Now, following my own work in Britain and that of RANZONI (1953) in California, 28 species of these Hyphomycetes are recognized divided amongst 18 genera.

Almost all these aquatic Hyphomycetes have been grown in pure culture on nutrient agar. There is variation from species to species, but the cultural behaviour of each is fairly constant. In most species the colony is compact with some low aerial growth. In some the colony is more or

less white (e. g. *Tetrachaetum elegans* Ing. and *Tetracladium marchalianum* de Wild.), in others it is dark or almost black (e. g. *Tricladium splendens* Ing. and *Anguillospora longissima* (de Wild.) Ing.). In nearly all species the colony on agar fails to produce spores, but when a strip of it is immersed in water, spores are produced in a day or two.

An outstanding feature of these fungi is that the spore is rarely of a conventional form. It is true that in *Margaritispora aquatica* Ing. the spore is more or less spherical and in *Dactylella aquatica* (Ing.) Ranzoni somewhat oval, but in most species it is either a branched structure with long divergent arms, or it is much elongated and with a curvature usually lying in more than one plane. Amongst the branched types the most frequent is that in which the spore consists of four long arms diverging from a common point. This I call the tetraradiate type and it is about this type that I shall have most to say. In all these aquatic Hyphomycetes the spore is hyaline.

A study of those species with tetraradiate spores shows that the developmental pattern is very different in the various species which, therefore, have to be distributed amongst a number of genera each characterized by a particular spore ontogeny.

In certain genera the tetraradiate spore is attached to its conidiophore by the tip of one of the four arms. This is true in the genera *Clavariopsis*, *Heliscus* (especially *H. longibrachiatus* Ing.), *Tetracladium* (especially *T. marchalianum* de Wild.), *Articulospora*, *Tetrachaetum* and *Alatospora*.

In *Clavariopsis aquatica* de Wild. and *Heliscus longibrachiatus* the first-formed arm of the spore is clavate with a blunt apex from which subsequently the other three thinner arms arise and grow out simultaneously. In *Clavariopsis* the spore is a terminal thallospore or aleuriospore (as occurs, for example, in such genera as *Helminthosporium* or *Nigrospora*) whilst in *Heliscus* it is a phialospore (as, for example, in *Penicillium* or *Trichoderma*).

In *Tetrachaetum elegans* Ing. and in *Alatospora acuminata* Ing. the spore consists of a long hypha bent in the middle thus forming two arms of the spore, the other two arising at the point of flexion, side by side and at the same time, to contribute the other two arms. The essential difference between these two genera is that the spore of *Tetrachaetum* is a terminal thallospore whilst that of *Alatospora* is a phialospore.

Tetracladium (with three described species) and *Articulospora* (also with three species) are both genera in which the spore is a terminal thallospore and the four arms arise in succession, but the details of development are different. In *Tetracladium* there are two or three knobs or finger-like processes in addition to the four arms.

Let us now turn our attention to certain other genera in which the tetraradiate spore is attached to its conidiophore near the point of divergence of the four arms. This is so in *Lemonniera*, *Triscelophorus* and *Actinospora*.

In *Triscelophorus monosporus* Ing. the spore is a terminal thallospore and its four arms arise in succession. In *Lemonniera aquatica* de Wild. the conidiophore is usually branched with a small group of phialides at its free end. Each of these produces a spore of four long divergent arms which arise and develop simultaneously. In *Actinospora megalospora* Ing. (INGOLD, 1952), as in *Lemonniera*, the conidiophore is branched, although the mode of branching is somewhat different, and the end-cell of each branch bears a large spore with four long divergent arms which make their appearance and develop simultaneously. However, in *A. megalospora* the spore is a terminal thallospore. This particular fungus has not been found on leaves but on submerged sticks in a stream in Britain. I have also found it recently in a river in Jamaica. In culture it differs strikingly from all other aquatic Hyphomycetes which I have grown. Growth on malt agar (and on other agars tested) is very sparse and no aerial hyphae are produced. However, the mycelium penetrates to a depth of 5-6 cm. below the surface of the agar. It looks as if the fungus is a microaerobe.

In the genus *Tricladium* (with four described species) the spore is a terminal thallospore and is more or less tetradiate. The spore consists of a filamentous main axis, twisted so that it does not lie in a single plane, and of two lateral branches which arise a short distance apart and point in different directions.

I have examined a large number of leaves from many localities in Britain and have described 25 species of these fungi. Of these 20 species are also known from Ireland as the result of observations by FENTON (1950) and myself. Nine species are also recorded from Switzerland (INGOLD, 1949). Further I have examined a small collection of submerged decaying leaves from New Zealand and on these nine aquatic Hyphomycetes have been identified all belonging to species known from Britain. Recently RANZONI (1953) has given the results of his investigations on these fungi as they occur in California. He found 17 species which I had also recorded in Britain and also described five new species including one belonging to a new genus (*Campylospora*). Five species (*Alatospora acuminata* Ing., *Flagellospora curvula* Ing., *Tetracladium marchalianum* de Wild., *Lemonniera aquatica* de Wild., and *Tricladium splendens* Ing.) have been reported from all five localities (i.e. Britain, Ireland, Switzerland, California, New-Zealand). It seems, therefore, that these fungi have a very wide distribution as well as being extremely abundant where they occur.

The new genus from California, *Campylospora chaetocladia* Ranzoni represents still another method by which the four-armed spore can be developed. It is a terminal thallospore rather like a desmid with two parts joined by an isthmus, one « semi-cell » having one and the other three bristles. The whole spore represents an extraordinary type of asymmetry. Before the appearance of RANZONI's paper, I had, together

with E. A. ELLIS, described certain peculiar spores from the scum of a freshwater tidal ditch in Norfolk (INGOLD & ELLIS, 1952). One type figured by us certainly belongs to *Campylospora*. Two other types represent still further methods by which the four-armed spore may be achieved.

Clearly the tetra-radiate spore would seem to have some biological value in the aquatic habitat. Possibly such a spore tends to remain suspended longer in the water and not to settle too soon. On the other hand it may act as an anchor and readily become entangled in a suitable substratum, for in a stream the coming to rest of a spore on a submerged leaf may present a real problem. It is interesting to note in passing that the tetra-radiate aquatic dispersal unit has been evolved indepently in certain species of the brown seaweed *Sphacelaria* where reproduction is often vegetatively by propagules. In some species these propagules bear an extraordinary resemblance to the tetra-radiate spores of aquatic Hyphomycetes.

My discussion of aquatic Hyphomycetes has been limited largely to those types with tetra-radiate spores. Some species have branched spores that are not of this nature, especially *Varicosporium elodeae* Kegel and *Dendrospora erecta* Ing. Again quite a number of species have spores which are long and worm-like. Two very common species may be mentioned in which the long filamentous spore is usually twisted into a sigmoid form lying in more than one plane: *Anguillospora longissima* (de Wild.) Ing. where the spore is a terminal thallospore and *Flagellospora curvula* Ing. where it is a phialospore.

My recent work on freshwater aquatic Ascomycetes was undertaken partly in the hope that these would have imperfect stages which might throw light on aquatic Hyphomycetes, but although a number of species have been grown in pure culture, no imperfect stages have been produced either directly on agar or when strips of an agar colony were submerged in water.

Submerged decaying leaves may also yield other Hyphomycetes — the areo-aquatic fungi. These have been investigated during the past few years particularly by van BEVERWIJK in Holland and by GLEN-BOTT in my own department. In the vegetative state they are typically submerged aquatic organisms growing especialy on water-logged leaves at the bottom of ponds — often stagnant ones. However, sporulation does not occur under these conditions, but only when the leaves are exposed to the air. In nature this might happen at the edge of a pond drying up in the summer. Most of the species involved belong to such genera as *Helicodendron*, *Helicoon* and *Clathrosphaerina*. The spores tend to be relatively large and complicated structures, but they become readily detached when mature. On a water surface they float and are difficult to submerge because of air trapped in the structure of the many-celled spore. It seems to me likely that in nature these spores are dispersed on the surface of water.

Although much is now known of the occurrence, structure and development of aquatic Hyphomycetes, much still remains to discover concerning their physiology and ecology. It would be interesting to know the range of organic substances which they can utilize, how their growth is affected by oxygen supply in the water, their temperature relationships, and how far they are responsible for the decay of leaves and other vegetable matter below water under natural conditions.

I have said nothing about the aquatic Hyphomycetes of the sea, but a number have been described. Special mention may be made of the work of BARGHOORN and LINDER (1944). In particular *Orbimyces spectabilis* Linder deserves special attention. This fungus was found on piling (probably oak) in Boston Harbour. The spore consists of a spherical part capped by four long divergent processes. It is a marine example of the tetraradiate aquatic spore.

A study of aquatic Hyphomycetes and Ascomycetes over the past twelve years has forced me to consider form and function in spores. No doubt in very many fungi there is no special survival value in the particular size and shape of a spore. Further it is always unsafe to deduce function from form. Nevertheless, when a comparison is made between the hyphomycete spores trapped in the scum of a stream and those caught by exposing sticky slides in the air, some explanation of the striking differences must be sought. It is all too easy to suggest an explanation. It is a very different matter to prove that the explanation is correct.

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4. — SEXUALITE DES ASCOMYCETES

RAPPORT

SEXUALITY OF THE ASCOMYCETES (*)

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In view of the extreme variation in the sexual process in plants and animals, it is not a simple matter to define *sexuality*. For purposes of the present discussion, it is considered that the essential features of a complete sexual process are karyogamy and meiosis. The Ascomycetes are very consistent as to where karyogamy and meiosis occur. With rare exception, they occur in the ascus. It is with regard to the manner in which compatible nuclei are brought together prior to karyogamy that these fungi show the greatest amount of variation in sexual reproduction. The methods by which plasmogamy is accomplished are extremely diverse.

The types of plasmogamy most commonly reported in the Ascomycetes (with typical examples of each) are: (1) fusion of two uninucleate cells or gametangia (Endomycetales), (2) fusion of antheridium and ascogonium, the latter usually provided with a trichogyne (*Ascobolus magnificus*, *Pyronema confluens*), (3) fusion of trichogyne with conidia or « spermatia » (a number of pyrenomycetes, discomycetes, and lichens; Laboulbeniales), (4) fusion of ascogonium with a copulation hypha (*Glomerella*, *Sordaria*), (5) somatogamy, or hyphal fusions (*Morchella*, *Tuber*). In addition it has been reported, but not adequately proven in most cases, that pairing of ascogonial nuclei without plasmogamy may occur in some species, or that plasmogamy may occur between two adjacent ascogonial cells. Various modifications of the above-mentioned methods are to be found. As yet there is not sufficient evidence to permit a proper evaluation of the various types of plasmogamy. Certain investigators consider the fusion of conidia or « spermatia », with a trichogyne as one of the most characteristic forms, and they find it indicative of phylogenetic relationships with the red algae. One might question whether there is yet sufficient evidence for such conclusions.

In the past it has been almost customary to consider as antheridium and ascogonium any two adjacent hyphal branches which show a mutual

* Bibliographical references to most of the investigations mentioned in this paper will be found in a recent publication by the writer (*Botanical Review*, 19, 439-586, 1953).

coiling and give rise eventually to ascocarps. Recent studies by the Louisiana State University group, investigating sexual reproduction in *Glomerella cingulata*, have shown that the perithecium is initiated by two such coiling branches, both uninucleate at origin. However, the outer initial proliferates profusely and forms the perithecial wall, while the inner one becomes a coiled ascogonium. A slender copulation hypha enters from the same hypha that bears the initials or from a neighbouring hypha and fuses with the ascogonium. There is reason to believe that a reinvestigation of certain other pyrenomycetes may reveal a situation similar to this. It may also be found that some species in which the ascogonium has been described as developing apomictically actually belong in this group and that the so-called « functionless antheridia » of certain authors are perithecial wall initials.

In the light of recent cytological and genetic investigations, the theories of HARPER and GWYNNE-VAUGHAN on double fertilization and an extra reduction division in the Ascomycetes are no longer acceptable. In our own laboratory we have found, with the aid of two different mutant genes affecting ascospore color, that segregation in *Ascobolus stercorarius* occurs in typical fashion in the first or second meiotic division and that there is no genetic evidence of brachymeiosis (George BISTIS, Thesis in progress, Columbia University).

Both homothallism and heterothallism are common in the Ascomycetes. DODGE discovered the first clear-cut case of heterothallism in the group in *Ascobolus magnificus*, and later he and others established the existence in *Neurospora* of a typical heterothallic condition controlled by a single allelomorphic pair of mating-type factors. WHITEHOUSE has pointed out that this bipolar type of heterothallism is characteristic of heterothallic Ascomycetes in general. In addition he has recognized two types of heterothallism: (1) physiological heterothallism, involving a differentiation into two compatible self-sterile strains showing no differences in sexual morphology, and (2) morphological heterothallism involving morphologically distinct male and female thalli. Most heterothallic Ascomycetes come under the first definition. A typical example of physiological heterothallism is found in *Ascobolus magnificus*, the ascospores of which give rise to cultures of two self-sterile mating types. As is probably characteristic of most heterothallic species, neither culture produces any sex organs when grown alone. But when the two mating-types are brought together on the same culture dish, each produces both ascogonia and antheridia. Plasmogamy occurs only between sex organs of opposite mating-types.

It must be admitted that examples of morphological heterothallism are difficult to find in the fungi. WHITEHOUSE has cited as examples of this group the dimorphic species of Laboulbeniales. The ascospores of these unusual species are discharged in pairs and upon infecting the host one gives rise to a male and the other to a female thallus. Nevertheless, this

does not necessarily mean that these species are genetically heterothallic. Since their thalli are much reduced, it is possible that each individual is no longer able to produce both male and female organs, as is characteristic of most species. It is also quite possible that the sexual differentiation of these paired thalli is a purely physiological phenomenon, with the thallus that begins to grow more vigorously becoming the female and the slower, smaller one becoming the male. Before a satisfactory appraisal of the sexual cycle can be made, it will be necessary to separate the ascospores of each pair and to study single-spore infections.

Recently, *Sclerotinia narcissi* was described by DRAYTON and GROVES as a morphologically heterothallic species. There are indications that the strains studied by these investigators have developed by natural mutation from a homothallic fungus. There is no real justification for the claim by these authors that only morphological heterothallism is synonymous with true heterothallism.

We have lately been studying in our laboratory the fungus, *Pericystis apis*, which we believe to be a good example of a morphologically heterothallic Ascomycete (Charles SPILTOIR, Thesis, Columbia University). As CLAUSSEN and others have already observed, the fungus is differentiated into male and female thalli. However, we do not find that these investigators have accurately described the sexual process. When male and female thalli are brought together, the female produces three-celled ascogonia, the terminal cell being a trichogyne. Contrary to previous claims there are no male organs. The trichogyne, upon coming into contact with the male hypha, fuses with it, and thus plasmogamy is accomplished. As long as male and female cultures are grown separately no sexual differentiation is apparent. It is therefore evident that even in such a distinct case of morphological heterothallism as this, it is necessary that the two sexes be brought together so that there can be a *physiological* interaction between them before the sexual nature of each thallus becomes apparent.

A number of investigations, mostly recent ones, have demonstrated that an ever-increasing number of Ascomycetes belong to a group which does not fit well into the concept either of homothallism or of heterothallism as originally conceived by BLAKESLEE. These fungi, even strains within the same species, show varying degrees of development between homothallism and heterothallism. Examples of this group are *Glomerella cingulata*, *Ophiostoma fimbriata*, and *Hypomyces solani*. In *G. cingulata* various perithecial and conidial cultures have been obtained from the wild-type through spontaneous mutation and segregation. Although certain perithecial cultures are truly homothallic when grown alone, any of the cultures may be crossed with one or more other cultures. It is also possible to cross two self-sterile strains with the resultant production of fertile perithecia containing heterozygous asci. From one such cross, typical homothallic cultures

are recovered among the progeny. Thus in *Glomerella cingulata* one may proceed from a homothallic to a heterothallic condition, or from heterothallic to homothallic behavior. Extensive genetic studies have shown that the mating reactions and phenotypes of the cultures are controlled by allelomorphs at two linked loci. All strains which have been studied cytologically have been found to have a haploid chromosome number of 4. There is reason to believe that the sexuality of *Hypomyces solani* will eventually be found explicable on a similar basis and that the claim of HIRSCH, HANSEN, and SNYDER that the differences among the so-called hermaphroditic, male, female, and neuter strains are explicable on the basis of differences in chromosome numbers will be disproven.

One unusual feature of the *Glomerella* findings deserves further attention here. When a conidial culture, which is completely self-sterile when grown alone, is crossed with a fertile perithecial culture, a considerable number of perithecia formed at the line of contact contain only asci homozygous for the conidial genotype. The available evidence indicates that the conidial strain, probably through some hormonal mechanism, is stimulated by its contact with the perithecial strain to produce perithecia of its own. This explains why such cultures can not be properly referred to as « males » and « females ».

It should be noted in passing that even in such typically heterothallic species as *Neurospora sitophila*, the characteristics of the two mating-types may be modified by other factors. For example, factors apart from those determining mating type have been found which control the presence or absence of perithecial primordia in single-ascospore cultures grown alone.

Until recently, the general consensus of opinion on homothallic fungi has been that they possess a sexually inferior type of life cycle with no opportunity for cross-karyogamy and recombination. Recent genetic studies of homothallic fungi have opened up a new approach to this problem and have already produced evidence that heterozygosity and recombination are common features of sexual reproduction in these as well as in heterothallic species. Naturally, one is not likely to observe these phenomena in the laboratory if he studies only isolated single-spore cultures of homothallic species. Such investigations, if carried no further, may fall far short of revealing the full potentialities for variation in the life cycle.

For example, *Sordaria fimicola* has generally been considered a typical homothallic fungus. But in 1942, GREIS, by means of irradiation experiments, was able to obtain sexual variants of this species, including certain self-sterile « males » and « females », which when crossed gave rise to fertile perithecia. Recently, we obtained a fully fertile mutant of *S. fimicola* with light ascospores. When the mutant culture is paired with the wild-type many crossed perithecia with heterozygous asci are obtained. These asci always contain four dark, normal and four light, mutant ascospores. Segre-

gation for the spores-color factor occurs in one-third of the asci in the first division and in two-thirds of the asci in the second division. Thus it is clear that two completely fertile cultures of a homothallic species, showing no differences in sexuality, may be freely crossed.

The cleistothecial *Aspergillaceae* have also generally been considered as typical homothallic fungi. But as early as 1926, DERX had reported the crossing of self-sterile strains of *Penicillium luteum* with the resultant production of fertile perithecia. HENRARD, in 1935, demonstrated cross-karyogamy in *Aspergillus nidulans* with respect to a physiological character. More recently, PONTECORVO and co-workers have demonstrated, with the use of biochemical and conidial-color mutants that cross-karyogamy and segregation occur readily in *A. nidulans*. Most of the strains used in crosses by these workers were fully self-fertile. Yet in certain crosses the number of asci showing cross-karyogamy in the line of cleistothecia formed between paired cultures approached 100 %, thus revealing preferential cross-karyogamy — a condition referred to by these investigators as « relative heterothallism ». The existence in nature of heterokaryoses in the mycelia of wild *Aspergillaceae* has also been reported. One might conclude from these observations that cross-karyogamy and segregation are probably of common occurrence in nature in the sexually reproducing species of the group.

One other outstanding feature of the interesting studies being conducted by the PONTECORVO group is the demonstration that karyogamy occurs on rare occasion in the mycelium of *Aspergillus*, regardless of the presence or absence of the sexual phase. This has been convincingly supported by a considerable quantity of genetic and cytological data supplied by these investigators. The practicality of the phenomenon in its application to obtaining recombinants in imperfect fungi — for example, *Penicillium notatum* — is readily apparent.

In conclusion, it should be emphasized that an imposing number of recent investigations have accumulated which demonstrate that the sexual process in the fungi is much more labile and subject to a great deal more variation than has commonly been realized. It is becoming increasingly clear that heterokariosis, heterozygosity and segregation may occur freely in homothallic fungi as well as in heterothallic fungi, and that it is even possible to obtain recombinants in imperfect fungi by means of rare somatic karyogamy and segregation. It is also apparent that many fungi are characterized by intermediate processes of sexual reproduction which range between the usual concepts of homothallism and heterothallism. In addition, there is further evidence in support of the concept that heterothallism has evolved from homothallism and that homothallism has also evolved from heterothallism independently and at various times in the fungi.

VARIATION IN THE HOMOTHALLIC FUNGUS *SCLEROTINIA TRIFOLIORUM* ERIKSSON

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INTRODUCTION

This work was undertaken to determine the extent of variation of the pathogen, and whether it might influence a programme of breeding for resistance to Clover Rot. Previous workers (1. 3. 4.) have established the fungus to be homothallic, and shown the existence of numerous biotypes.

MATERIAL AND METHODS

Nine wild isolates were collected, seven from red clover, one from beans and one from sainfoin. From these, 25 sets of 8 spores from individual asci were isolated, approximately three from each isolate. Inoculum for the pathogenicity tests was dried mycelium in bran (2). It was dusted over the plants maintained in a humid atmosphere. Growth rates were measured in long glass tubes of 8 mm. bore half-filled with potato-dextrose agar and incubated at 20° C. after inoculation at one end with the fungus (6).

EXPERIMENTAL DATA AND RESULTS

Variation between wild isolates.

When the seven isolates from red clover were inoculated on to this host a range of pathogenicity was recorded. The most pathogenic isolate and the least plus those from beans and sainfoin were later inoculated on to all three hosts. The order of pathogenicity, which did not vary from host to host was (1) Red Clover Isolate I (2) Sainfoin Isolate (3) Bean Isolate (4) Red Clover Isolate II. The growth rates of all nine isolates were measured. These varied significantly, and were inversely correlated with pathogenicity.

Heterogeneity of the wild isolates.

When monoascospore lines of different genotype are co-inoculated on the same agar plate a white « reaction » line of microconidia is formed where the two mycelia meet. This is absent when both co-inoculants are of the same genotype (1). This character was used to determine whether the isolates were heterocaryons by co-inoculating the ascospore progeny. Three were heterocaryons and the remainder homocaryons.

Segregation studies.

LOVELESS (5) examined the segregation of reaction-types in Var. Fabae, and found that each ascus contained either all eight of one reaction-type or four of each of two types. Diallel co-inoculations were performed for each ascus set isolated from the three heterocaryons. In each case all four products of meiosis were found to be of different reaction-type. When the growth rates were measured segregation was into four groups of two for some ascus sets. For others it was into two groups of four or one of four and two of two. Each ascus set was inoculated on to red clover, and pathogenicity was found to be inversely correlated with growth rate. Other segregations noted include two sizes of ascospores arranged in first division pattern, and two cultural types. In one, sclerotia were in abundance with few microconidia, whilst in the other microconidia were the more abundant. Ascus sets from the six homocaryons did not segregate for any of these characters.

The mechanism of heterocaryosis.

A plant sprayed with spores of two reaction-types formed heterocaryotic sclerotia at the base. Sclerotia removed from co-inoculation plates of the two cultural types were all heterocaryotic even though microconidia were absent from some of the plates. On examination of the reaction line anastomoses between hyphae of the two genotypes were discovered. Heterocaryosis in culture therefore appears to occur by nuclear migration across the points of anastomosis. Microconidia appear to be functionless.

Properties of heterocaryons.

When the growth rates of heterocaryons were compared with those of their constituent homocaryons some grew at a greater speed. Others were slower than either. Measurements of pathogenicity yielded a similar result.

Behaviour of nuclei at nuclear fusion.

A heterocaryon was produced from two single spore lines from a large and a small ascospore. Apothecia were sectioned and a thousand asci examined for the presence of all large spores, all small, or four of each. A ratio of 1 : 1 : 2 was consistent with fusions of the two types of nuclei having occurred at random.

Segregation of new biotypes.

Three ascus sets were isolated from a heterocaryon of two reaction

types, and each was back co-inoculated. Two sets reacted with both parent cultures, and of the third, four reacted, two showed no reaction with one parent, and two showed no reaction with one parent, and two showed no reaction with the other. Several new biotypes had segregated from this cross.

DISCUSSION

The evidence from cultural studies suggests that heterocaryons probably arise on the plant when lesions from ascospores of different genotype run together. Complications in the breeding programme are likely to arise from:

1. The immense number of new biotypes which may segregate even when heterocaryons arise in only one third of cases.
2. The greater virulence of certain heterocaryons, but only if these remain stable for a sufficient length of time before segregating.

There is however, no evidence of biologic races.

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UEBER DIE SEXUALITÄTSVERHÄLTNISSE DES ASKOMYZETEN BOMBARDIA LUNATA ZCKL

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Bombardia lunata Zckl. ist zwittrig und selbststeril. Es gibt zwei Reaktionstypen: A und a. Fruktifikation und Sporenbildung findet nur zwischen Stämmen der komplementären Reaktionsgruppen (A und a) statt. Die Fähigkeit der Fruktifikation ging bei sämtlichen seit Anfang der 30-er Jahre in Kultur befindenden Einsporstämmen verloren, blieb jedoch in der Mischkultur erhalten.

Die aus der Mischkultur neu gewonnenen Einsporstämme sind männlich und weiblich fertil, sie bilden auf beiden Seiten Perithezien.

Bei Kreuzungen von Neustämmen mit Altstämmen zeigen sich die

Altstämme männlich fertil, jedoch weiblich steril. Perithezien finden sich nur auf der Seite des Neustammes. Fruktifikation wird nur bei Spermatisierung neuer Stämme mit Spermarien von Altstämmen, jedoch nicht umgekehrt beobachtet.

Ein Teil der Neustämme wurde schon nach 1 1/2 Jahren wieder weiblich steril, blieb aber männlich fertil. Die im Laufe der Passagen erworbene weibliche Sterilität wird bei Kreuzung mit weiblich fertilen Stämmen nicht vererbt; alle aus Sporen solcher Kreuzungen gezogene Myzelien sind weiblich fertil.

Es müssen hormonale Einflüsse zwischen Komplementärmyzelien existieren, die das weiblich Sterilwerden, wie es in Einsporkulturen nach einer Reihe von Passagen stattfindet, verhindern. Komplementäre Myzelien üben auch einen beschleunigenden Einfluss auf die Entstehung von Spermogonien aus.

Résumé. — *B. lunata* Zckl. est hétérothallique; il présente cependant deux types de réactions (A et a) qui sont intrastériles mais interfertiles. Après une série de repiquages, les cultures monospores deviennent stériles en ce qui concerne les femelles, fertiles en ce qui concerne les mâles; en cultures mixtes la fertilité femelle ne se perd pas, cette survivance de la fertilité femelle est fondée sur une interaction hormonale des mycéliums complémentaires. C'est également grâce à de semblables influences que se trouve favorisée l'apparition de spermogonies.

LA NEOTENIE CHEZ LES ASCOMYCETES

par Fernand et Mme MOREAU

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Le développement des périthèces lagynomorphes de beaucoup d'Ascomycètes comporte un ascogone qui, sous la protection d'une enveloppe issue de la base de l'ascocarpe, donne naissance à des hyphes ascogènes produisant les asques.

Chez les *Pleurage anserina* et *setosa*, le *Chaetomium elatum*, le *Magnusia nitida*, le *Microascus Doguetii*, on observe fréquemment un certain nombre de fructifications anormales, à cycle raccourci: le périthèce demeure petit et reste clos, la formation d'hyphes ascogènes étendus y est réduite ou même supprimée et les asques produits y sont peu nombreux.

A un stade plus ou moins précoce, des cellules fertiles binucléées grossissent, se montrent le siège d'une caryogamie et se transforment en asques; pareille transformation peut atteindre soit des cellules d'hyphes

ascogènes soit les cellules ascogoniales elles-mêmes. De tels phénomènes se font sans que l'enveloppe périthéciale ait acquis les caractères qui sont les siens au moment de la formation bien plus tardive des asques dans les périthèces habituels.

Ces périthèces hâtifs, dont le développement des parties fertiles rompt le synchronisme qu'il présente ordinairement avec celui des parties stériles, et qui fournissent des asques dès l'état juvénile, qu'ils ne dépassent d'ailleurs pas, peuvent être dits néoténiques: leur production est comparable aux cas de néoténie connus dans le règne animal et chez les végétaux supérieurs.

Un *Pyrenomycète* atteint de néoténie présente un développement de son périthèce qui n'est pas sans rappeler celui d'un périthèce d'*Erysiphales*: chez elles, sous une enveloppe périthéciale close, des cellules ascogoniales deviennent directement des asques ou donnent naissance à des hyphes ascogènes d'étendue restreinte qui forment les asques. On est ainsi amené à accorder aux *Erysiphales* la valeur d'un groupe néoténique.

A TENTATIVE SCHEME OF SEXUAL REPRODUCTION IN *PYRONEMA OMPHALODES* (BULL. EX FR.) FUCKEL (= *P. CONFLUENS* PERS. EX TUL.)

by Irene M. WILSON

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This paper is an attempt to synthesise the most fully substantiated facts in the conflicting accounts of sexual reproduction in *Pyronema*. It is based on the literature (cited in WILSON, *Annals of Botany*, N.S. 16, 1952), on my own published and unpublished work and on a general consideration of the cytology and genetics of other Ascomycetes.

Accounts of a failure of copulation between the antheridium and trichogyne of *Pyronema* may be due to cultural conditions, especially to deficiency of light (ROBINSON 1926, KERL 1937, WILSON 1952). It is well established that, except in var. *inigneum*, an open communication is formed between the antheridium and trichogyne and that the antheridium eventually loses most of its contents. Figures by HARPER (1900) and CLAUSSEN (1912) claiming to show the complete breakdown of the wall at the base of the trichogyne to allow the entry of male nuclei into the ascogonium have been justly criticised by MOREAU and MOREAU (1930) and others. Such criticisms may be irrelevant to the real issue since BULLER (1931, 1933) has drawn attention again to the septal pore and its importance in the translocation of cytoplasm and perhaps also of nuclei.

I find that ascogenous hyphae may start to develop 1-2 hours after the fusion of the trichogyne with the antheridium, but I have so far failed to see the passage of a male nucleus through the septal pore during this period. Nor has this been recorded by any author. More observations need to be made of this critical period. Later, when ascogenous hyphae are forming, food material passes through the pore.

The possibility that plasmogamy may have occurred at an earlier stage by hyphal fusions and that the antheridium has lost its sexual function (as maintained by the Dangeardian school) must also be considered. Hyphal fusions, though not a conspicuous feature, can be found both in single-spore and multi-spore cultures, especially in unfavourable conditions. *P. omphalodes* is homothallic and, in single-spore culture, apart from the possibility of mutation, all the nuclei in the hyphae and sex organs must be alike. In multi-spore cultures, probably more frequent in nature, the mycelia are intermingled and either the union of sex organs (which arise as dichotomies of different hyphal branches) or previous hyphal fusions might well result in the association in the ascogonium of nuclei of different genotype, the main object of plasmogamy.

Ascogenous hyphae arise opposite pairs of nuclei (CLAUSSEN 1912) or groups of nuclei (WILSON 1952) and the nuclei pass one by one into them. A basal bulb is formed and from this arise one or more narrower branches in which pairs of nuclei have been seen by most workers near the tips. According to CLAUSSEN (1912) the nuclei divide conjugately *i.e.* with parallel spindles, and a series of binucleate cells is formed. CLAUSSEN's fig. 43 of this division shows only one pair of nuclei and the hypha is not shown attached to an ascogonium so that there is no proof *from the figure* that this was an ascogenous hypha at all. There is reason to doubt whether true conjugate mitosis occurs in *Pyronema*. GWYNNE-VAUGHAN and WILIAMSON (1931) have found a simultaneous division of the nuclei in single file in the branches and I have confirmed this (WILSON 1952), but the former authors consider that the nuclei are diploid and I do not. Walls are formed across the spindles and the resulting cells have 1,2,2...1 nuclei. The terminal cell does not develop further. If grown in bright illumination, the top of the primary ascogenous hypha is hooked like a crozier, so that it may sometimes be cut away in microtomed sections.

The top binucleate cell and some of the others form croziers in which the nuclei again divide simultaneously in single file and walls are formed across the spindles to give cells with 1,2,1 nuclei. The penultimate binucleate cell forms an ascus or another crozier. The uninucleate end cell usually fuses with the uninucleate stalk cell and the binucleate cell so formed grows out to form another crozier. By repetition of this process a succession of asci can be formed.

It is suggested that wherever two nuclei of different genotype are

associated growth may be stimulated. This would explain 1) the selection of *paired* nuclei to enter the ascogenous hyphae, 2) the isolation of pairs of nuclei in the upper parts of the primary ascogenous hyphae, 3) the outgrowth of binucleate cells to form croziers, 4) the growth of an ascus or a crozier from the penultimate cell and 5) proliferation.

It is agreed by all observers that the two nuclei in the young ascus fuse together. As regards the ascus divisions controversy has centred on whether there is one reduction or two. HIRSCH (1950) using the modern « smear » technique which permits the use of whole asci has found 12 bivalents in the first division, 12 chromosomes in the second and 12 in the third, thus confirming CLAUSSEN (1912) and MOREAU and MOREAU (1931). I agree with this result and believe that accounts of lower chromosome numbers in the older work are due to the necessity, at that time, of using stains like Heidenhain's haematoxylin which required microtomed sections. If it is accepted that the nuclear divisions in the ascus consist of a meiosis occupying two divisions followed by a mitosis, there can be no earlier sexual fusion in the ascogonium.

COMMUNICATIONS DIVERSES

PARASITISME
DE *CLITOCYBE TABESCENS* (FR. EX SCOP.) BRES.
SUR *COFFEA CANEPHORA* PIERRE

par G. BOURIQUET

Division de Défense des Cultures du Ministère de la France d'Outre-Mer

et H. BARAT

Laboratoire de Pathologie Végétale à Tananarive

Nous avons trouvé les fructifications de ce Champignon, auteur d'un pourridié qui sévit dans les plantations de Caféiers de la côte Est à Madagascar. L'aspect clinique rappelle beaucoup celui qui est déterminé par l'*Armillaria fuscipes* Petch (root *splitting disease*, rhizomorphes sous-corticaux et externes, absence de rhizomorphes libres dans le sol). L'absence d'anneau sur le pied a amené à le rattacher à *Clitocybe tabescens*. Certains caractères pourront toutefois justifier sa distinction comme une variété nouvelle. La question reste à l'étude. Description : « *Fructifications naissant en touffes serrées sur les craquelures des écorces de la plante hôte. Carpophores jeunes brun foncé très pelucheux à chapeau concave au milieu, à bords pleinement déroulés* dm. 1-8 cm. Centre brun foncé s'atténuant en dégradé jusqu'au blanc crème. Nombreuses écailles brunâtres, dispersées de façon homogène sur toute la surface du chapeau qui est strié par des saillies à l'emplacement des lamelles, et, sur les bords, par des saillies plus courtes à l'emplacement des lamellules. Pied cylindrique, fibreux, strié dans le prolongement des lamelles, brun noir à sa base parfois épaissie, de couleur claire variable dans sa partie haute; dm. 3-8 mm, parfois 12 mm à la base. Pas d'anneau. Lamelles blanches adnées ou très légèrement décurrentes; lamellules entre chaque lamelle sur 1/3 à 1/2 du rayon. Souvent une 2^e lamellule très courte entre 2 lamelles. Basides en massue (29-40) $\times$ (9-10) μ . Basidiospores blanches, ovales ou presque rondes (6-11) $\times$ (4,4-6,5) μ . Hyménium homomorphe ». Hôtes connus : *Albizia stipulata* Boiv., *A. Lebbeck* Benth., *A. moluccana* Miq., *Leucaena glauca* Benth., *Tephrosia candida* DC., *Inga affinis* DC. (= *I. dulcis* Mart.) (rare), *Aleurites Fordii* Hemsl., *Coffea canephora* Pierre (var. Kouilou très sensible et var. *Robusta* plus résistante), Agrumes, Bananier, Kapokier, Giroflier, une Solanée arborescente. Le mycélium est luminescent dans la nature et dès la germination des spores en culture pure.

A REVISION OF THE CLASSIFICATION OF OPERCULATE DISCOMYCETES (PEZIZALES)

by Richard P. KORF

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A new classification of Operculate Discomycetes is proposed recognizing three families: (1) Cyttariaceae, (2) Sarcoscyphaceae, (3) Pezizaceae. The characters used in this revision are, in order of importance, those of: (1) the ascus, (2) the ascospores, (3) the tissue structure of the apothecium, (4) microchemical reactions, particularly that of iodine, (5) gross morphology, (6) type of sex organs, (7) conidial states. This is a departure from the older systems (e.g., LINDAU, REHM, BOUDIER) based almost wholly on gross morphology. Eleven tribes are recognized in the Pezizaceae based on these characters: (1) Pezizeae (= Aleuriées *sensu* BOUDIER p. max. p.), (2) Ascoboleae, (3) Discineae, (4) Rhizineae, (5) Psilopezizeae, (6) Morchelleae, (7) Gyromitreae (*sensu* NANNFELDT), (8) Acetabuleae (*sensu* Nannf.), (9) Ciliarieae, (10) Octosporeae (= Humarieae Auct.), (11) Pyronemeae (= Pseudo-Ascoboleae *sensu* LE GAL p. max. p.).

Investigations of genera with very small apothecia reveal that these are polytypic in some cases. *Ascophanus* Auct. must be divided into three genera: (1) *Ascophanus* Boud. emend. (Pezizeae), (2) *Coprotus* n. gen. (Acetabuleae), (3) *Ascophanella* n. gen. (Pyronemeae). *Jafnea* n. gen. (Ciliarieae, in honor of J. A. F. NANNFELDT) is also proposed and characterized.

THE CLASSIFICATION OF THE ECCRINALES, FUNGI INCERTAE SEDIS, AND THEIR DESCRIPTIVE TERMINOLOGY

by G. Safford TORREY

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The Eccrinales are Thallophytes which occur in the hind gut of various Arthropoda, attached to the chitinous lining. They have been regarded as Phycomycetes, but they may be better placed as apochromatic algae or in a new division. The existing knowledge has been set forth in

a paper (1) in which 41 spp. and 2 doubtful ones have been placed in 4 families and 12 genera, one of which is said to be probably reducible to synonymy.

I suggest some *substitutions* for the descriptive terms ('—') employed. 'Macroconidies' and 'microconidies', endogenous spores, are *aplanospores*, and may be qualified as *discoïd*, *isodiametric*, or *elongate*. 'Spores durables', are either 'conidiospores', that is *resting aplanospores*; or 'oospores', 'telospores', produced sexually or apogamously, that is *zygospores* or *azygospores*. The 'gland' (literally *glans*, from its shape), better *appendage*, is the body of the aplanospore from which a new filament has arisen. This is sometimes carried up by intercalary growth between the spore and the point of attachment, the new filament being formed at an angle with the axis of the spore. Or (appendage absent) the aplanospore may stretch directly into the filament. A terminal arthrospore on such a filament has been called a 'gland fertile', but is quite different from the appendage.

The differentiating characters of the families as given by the authors may be summarized as follows, my comments being in parentheses:

Resting spores 4-nucleate (31 spp., but resting spores unknown in 18).

On Myriapoda or Insecta; appendage absent — *Eccrinaceae*.

(An appendage is present in *Enterobryus elegans* Leidy, the oldest species).

On Amphipoda or Isopoda; appendage present — *Eccrinellaceae*.

(An appendage is reported in only 2 of the 4 spp. These two families are indistinguishable except by their hosts).

Resting spores 1-nucleate; on Amphipoda, Isopoda, or Decapoda —

Taeniellaceae. (Resting spores have been seen in 9 of the 10 spp., but in one these the number of nuclei is not stated, and in another the spore often becomes binucleate at or shortly after discharge).

Resting spores 2-nucleate, appendiculate; on Paguridae — *Arundinulaceae*. (Resting spores are unknown in 2 of the 3 spp.).

Neglecting the numerous cases where the differentiating characters are unknown, one may well question the significance either of the appendage, or of the number of nuclei in the resting spore. One is a detail of germination; the other depends on the point in a series of changes at which the spore rests, or at which it was observed. For comparison one might reflect on the value of similar characters in other groups. Should one erect families in the Peronosporales on the direct or indirect germination of conidiosporangia, or split the Zygnemaceae because some zygospores rest in the 1-nucleate and others in the 4-nucleate condition?

(1) DUBOSQ, O., L. LÉGER, et O. TUZET. Contribution à la connaissance des Eccrinides. Les Trichomycètes. *Arch. Zool. exp. et gén.*, 86, 29-144, 1948.

There seems, then, no reason to maintain more than a single family in Eccrinales. It may be characterized as

ECCRINACEAE Léger et Duboscq, *Arch. Anat. Micr.*, 25, 323 (1929), emend. Thallophyta pede substrato adfixa, tubulata, non chlorophyllosa, nucleis perspicuis, primum non septata, deinde arthrosporas v. aplanosporas saepe multiformas, demum in exuviis hospitium zygosporas v. azygosporas in nonnullis formantia. In integumento chitinoso Arthropodorum, saepissime in intestino posteriore. Gen. typ. *Eccrina* Leidy. Incl. *Taeniellaceae* et *Arundinulaceae* L. & D., l.c., et *Eccrinellaceae* D., L., et Tuzet, *Arch. Zool. exp. gén.*, 86, 83 (1948).

It is possible that *Amoebidiaceae* L. & D., should also be included. This would require some further emendation, however.

In the characterization of the genera of *Eccrinaceae* L. & D., emend., host relationships have been largely relied on. Whatever may have been the practice in parasitology, mycologists are not now inclined to give much weight to such characters where other distinctions are lacking, regarding similar forms on different hosts as congeneric, and morphologically identical ones merely as *formae speciales*. On this basis, not more than two or three genera can be maintained. A considerable number of species, must, however, be retained, at least until more life-histories are completely known, and the host-ranges of species are experimentally determined.

II. Physiologie des champignons et des végétaux hétérotrophes

I. — LES PIGMENTS DES CHAMPIGNONS : LOCALISATION, CYTOCHIMIE ET RÔLE

RAPPORT

THE PIGMENTS OF THE LOWER FUNGI (MOULDS)

by George SMITH

London School of Hygiene and Tropical Medicine

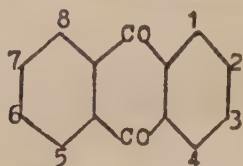
Colour in fungi has always been regarded as of taxonomic significance. In the higher Basidiomycetes species are distinguished according to the colour of the pileus, or, in some cases, the colours developed when the flesh is bruised. In the lower fungi, commonly known as moulds, conidial colours, mycelial colours, and pigments diffusing into the substrate are all of importance in classification. It is natural that chemists and biochemists should have turned their attention to some of these striking pigments, in the first place to determine their chemical structures, and secondly to attempt to elucidate the rôle they play in fungal metabolism.

The pigments of known constitution which have the simplest chemical structure are derivatives of benzoquinone. Fumigatin, 3-hydroxy-4-methoxy-2:5-toluquinone, is produced by a strain of *Aspergillus fumigatus* Fres. (1). Spinulosin, which is 6-hydroxyfumigatin, is produced by another strain of the same species, but was first obtained from a number of strains of *Penicillium spinulosum* Thom. (2). Two compounds, each built up from two molecules of a toluquinone, are phoenicin and oosporëin. The first was obtained by FRIEDHEIM (3) from *Penicillium phoeniceum* van Beyma and, later, by POSTERNAK (4) from *P. rubrum* Stoll. Oosporëin was isolated by KOEGL and VAN WESSEM (5) from *Oospora colorans* van Beyma, but has recently been isolated also from *Verticillium psalliotae* Treschow, a species of *Chaetomium*, and two unidentified sterile moulds. The two compounds are related in exactly the same way as fumigatin and spinulosin.

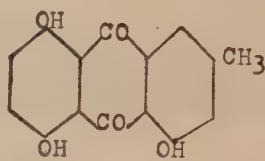
Naphthoquinones are rare and, so far, only two compounds have been reported, javanicin and oxyjavanicin, both from *Fusarium javanicum* Koor- ders. The two are closely related but, so far, their constitutions have not been completely determined (6).

Antraquinones are widely distributed, being produced by moulds belonging to several different genera. The first to be isolated in the

L.S.H.T.M. laboratories was helminthosporin, 2-methyl-4: 5: 8-trihydroxyanthraquinone, obtained first from the mycelium of *Helminthosporium gramineum* Rabenh. (7), and later from *H. catenarium* Drechsler, *H. tritici-vulgaris* Nisikado, and *H. cynodontis* Marignoni (8, 9). As an aid to following the subsequent argument, the formula of anthraquinone, numbered, and that of helminthosporin, are given below.



Anthraquinone

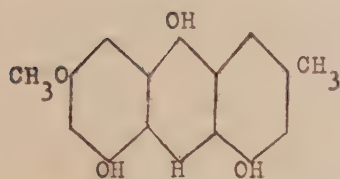


Helminthosporin

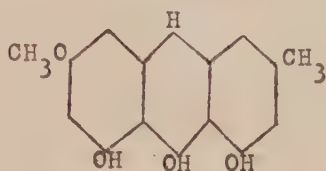
Cynodontin, which is 1-hydroxyhelminthosporin, is produced by *H. cynodontis*, *H. euchlaenae* Zimmermann, and *H. avenae* Eidam (8, 9). Isomeric with helminthosporin is islandicin, 2-methyl-1: 4: 5-trihydroxyanthraquinone, isolated from cultures of *Penicillium islandicum* Sopp (10), whilst catenarin, from *H. catenarium* (8), is 7-hydroxyislandicin, and erythroglaucin, obtained from a number of species in the *Aspergillus glaucus* series (11), is 7-methoxyislandicin. Tritisporin, another product of *H. tritici-vulgaris* (8), is related to catenarin but has a $-\text{CH}_2\text{OH}$ group in place of the $-\text{CH}_3$.

A number of pigments of this type are closely related to *Frangula* emodin, which is 2-methyl-4: 5: 7-trihydroxyanthraquinone. The latter substance itself has not yet been isolated from any species of mould, although it has been obtained by KOEGL (12) from the sporophores of *Cortinarius* (*Dermocybe*) *sanguineus*. Phiscion, the 7-methyl ether of emodin, is found in various lichens, and is also produced by several species of the *Aspergillus glaucus* series (11). Emodic acid and ω -hydroxyemodin have been obtained from a strain of *Penicillium cyclopium* West. (13), and the latter substance from *P. citreo-roseum* Dierckx (= *P. cyaneo-fulvum* Biourge) (14). The 4-methyl ether of ω -hydroxiemodin was obtained independently as carviolin by HIND from *P. carmino-violaceum* Dierckx (11), and as roseo-purpurin by POSTERNAK from *P. roseo-purpureum* Dierckx (16) (the two species are now regarded as synonymous).

One of the simplest of the substituted anthraquinones, and one which may be regarded, on paper at any rate, as the parent of all the rest, is chrysophanol, 2-methyl-4: 5-dihydroxyanthraquinone. It has recently been shown to be one of a complex mixture of pigments produced by *Penicillium islandicum*. Two other substances, which are of particular interest when considering the rôle of these pigments, are the two reduction products of physcion, physcion anthranols A and B, produced, along with other pigments, by some species of the *Aspergillus glaucus* series (11).



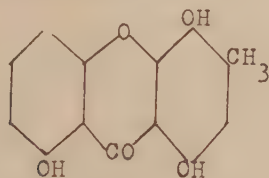
Physcion anthranol A.



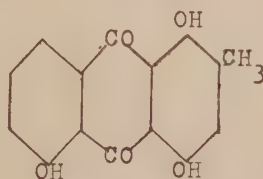
Physcion anthranol B.

Still more complex molecules are built up by a number of species of moulds. These substances have very high melting-points and some at least of them are most probably di-anthraquinones. Penicillipsin, obtained from *Penicillipsis clavariaeformis* Solms-Laubach, has the empirical formula $C_{30}H_{20}O_8$ (17), whilst a pigment which has been isolated from *Penicillium islandicum*, *P. wortmanni* Klöcker, and *P. rugulosum* Thom, and which is still under investigation, has the formula $C_{31}H_{20}O_{11}$. Rugulosin, the main pigment in the mycelium of *P. rugulosum*, is not an anthraquinone but is related in structure, since it gives, on thermal decomposition, some 30 per cent of a mixture of chrysophanol and emodin. Another of the pigments of *P. islandicum*, skyrin, is definitely a di-anthraquinone, the molecule consisting of two emodin molecules linked through either the 4 or 5 positions. On reduction with $Na_2S_2O_4$ it gives, almost quantitatively, two molecules of emodin. (18). The same substance, under the name endothianin, has been isolated by SHIBATA et al from the chestnut blight fungus *Endothia parasitica* Fr. (19). Still another complex substance is aurofusarin, produced by *Fusarium culmorum* W.G. Smith; it does not melt up to 360° and has the empirical formula $C_{30}H_{20}O_{12}$ (20).

Also related in chemical structure to the anthraquinones are the xanthenes, two of which have been found as metabolic products of moulds. Ravenelin, from *Helminthosporium ravenelii* Curtis and *H. turcicum* Pass., is a methyltrihydroxyxanthone, with the substituents in the same positions as in islandicin (21). Rubrofusarin, from *F. culmorum*, is a monomethyl ether of an isomer of ravenelin, but its constitution is not known with certainty (20).



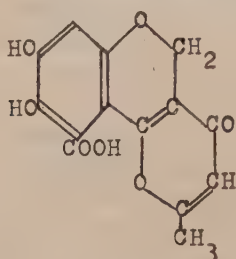
Ravenelin



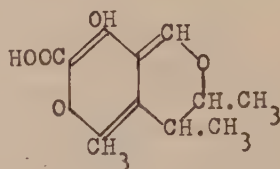
Islandicin

Apart from the quinones and related compounds there are several mould pigments which are of interest on account of distribution or unusual

properties. Citromycetin is a yellow substance produced by most strains of *Penicillium frequentans* Westling (22). It has also been obtained, in very good yield, from one strain of *P. roseo-purpureum*. Its complete chemical structure has only recently been determined by ROBERTSON *et al* (23, 24). Although not closely related to other mould pigments it shows affinities with a number of colourless mould products. Another substance which was first isolated and described in 1931 (25), but whose constitution has only recently been solved (26, 27), is citrinin, produced by practically all strains of *Penicillium citrinum* Thom, by one strain of *Aspergillus terreus* Thom, and by *A. niveus* Blochwitz. It is a golden yellow crystalline compound, antibacterial and also strongly antifungal.



Citromycetin

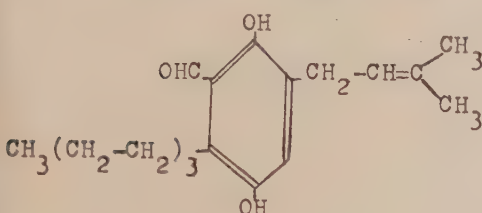


Citrinin

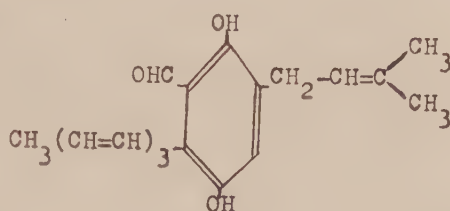
There are two known mould pigments which are unusual in containing chlorine. Sclerotiorin is an orange-yellow substance first obtained from *Penicillium sclerotiorum* van Beyma, being mainly responsible for the brilliant colour of the sclerotia of this species (28). It has the empirical formula $C_{21}H_{23}O_5Cl$ but its constitution has not been finally determined. Recently it has been isolated from the mycelium of *Penicillium multicolor* G.-M. et P. (29). The second chlorine-containing compound is nalgio-laxin, $C_{18}H_{15}O_6Cl$. This occurs, along with a second pigment nalgiovensin, $C_{18}H_{16}O_6$, in the mycelium of *Pen. nalgiovensis* Laxa. Both are anthraquinone derivatives, nalgio-laxin being monochloronalgiovensin, and both are optically active, due to a hydroxypropyl side-chain in position 2. Not only are optically active anthraquinones very rare, but this is the first recorded instance of a naturally occurring chloroanthraquinone (30).

In the three series of *Aspergillus* species which are regularly ascosporic the perithecia in the *A. glaucus* series are yellow to orange, in the *A. fischeri* series colourless, and in the *A. nidulans* series dark purple. A number of pigments which have been isolated from members of the *A. glaucus* series have already been mentioned. Two other pigments are of interest since they are most probably responsible for the colour of the perithecia. Flavoglaucin apparently occurs in all the perithecial species of this series, whilst auroglaucin is of frequent occurrence but is not elaborated by all species. Both are highly substituted quinols, and they

differ only in that flavoglaucin has a normal heptyl side-chain where auroglaucin has an unsaturated heptatrienyl chain (31, 32).



Flavoglaucin



Auroglaucin

The pigment responsible for the intense colour of the perithecia of *A. nidulans* and related species has only recently been isolated at L.S.H.T.M. (unpublished). It has been named asperthecin and is a hydroxymethyl-pentahydroxy-anthraquinone. The colouring matter is intercellular and cannot be recovered by extraction of the dried mycelium, as is usually the procedure. Instead the wet mycelium is suspended in dilute acid, disintegrated in a high speed macerator, filtered off but not dried, and then extracted with a large volume of ethanol.

The biological significance of mould pigments

There has been a widespread tendency to regard these substances as end products, or waste products, of metabolism. In a well-known book on the biochemistry of fungi pigments are entirely ignored, as of no practical importance. Against this view there is now a considerable amount of evidence.

1. Some pigments are produced in very large amounts, up to at least 30 per cent of the total dry weight of the mycelium.

2. In many cases the maximum content of pigment is reached whilst there is still available an appreciable amount of readily utilizable carbohydrate. Delay in harvesting cultures results in the gradual disappearance of the pigment as autolysis sets in.

3. The same pigment is frequently produced by two or more different species, often belonging to different genera or families. Several examples have already been given. This suggests that different species have solved a problem in the same way, rather than that they have produced the same substance as waste product.

4. The majority of the pigments which have been isolated and characterized are quinones. Most of these substances are readily and reversibly reduced to the corresponding quinols. It is not surprising that, in a number of cases, it has been shown that the quinol occurs along with the quinone. Thus fumigatin, from *Aspergillus fumigatus* is accom-

panied by 3-hydroxy-4-methoxytoluquinol; physcion occurs along with its two reduction products, the anthranols; phoenicin is accompanied by its leuco derivative, and FRIEDHEIM has shown (3, 34) that traces of the pigment increase enormously the respiration of unpigmented, washed cells of *Bacillus pyocyaneus*; *Helminthosporium leersii* secretes the yellow luteoleersin, $C_{26}H_{35}O_7$, along with the colourless alboleersin, $C_{26}H_{40}O_7$ (33); *Oidiodendron fuscum* Robak produces an orange coloured substance fuscine, $C_{15}H_{16}O_5$ together with dihydrofuscine, $C_{15}H_{18}O_5$ (35).

5. Anthraquinones are the commonest of mould pigments, yet, although this group of products contains many different substances, they are all similar in certain respects. In all of them the only carbon-containing substituent, i.e. $-CH_3$, $-CH_2OH$, $-COOH$, etc., is in position 2, and all of them have hydroxyl groups in positions 4 and 5. This is very striking and can hardly be a matter of chance.

6. Several of the pigments mentioned above are antibiotics, being both antibacterial and antifungal. It is at least possible that they have been elaborated as a defence mechanism against the encroachment of other organisms.

Pigments and taxonomy

Early work on the metabolism of moulds suggested that many of the substances isolated, both pigments and colourless compounds, were produced only by individual species, and that the detection of their presence might prove a useful aid in taxonomy. This hope has not been fulfilled. There are now many instances, some of them cited above, of substances of unusual chemical constitution being produced by several different fungi. On the other hand, morphologically similar moulds, which would be classed by the taxonomist as strains of the same species, often show surprising biochemical differences. One strain of *Penicillium cyclopium* gives 6-hydroxyemodin and emodic acid, another produces penicillic acid, whilst most strains produce neither. Fumigatin and spinulosin are produced respectively by two different strains of *Aspergillus fumigatus*. *A. terreus* shows the most bewildering diversity as regards biochemical characteristics of different strains, one strain giving citrinin plus terrein, another terrein alone, a third neither of these substances but itaconic acid, another succinic acid along with large amounts of fat, and so on. *Penicillium islandicum* is a morphologically well-marked species; five out of six strains which have been examined produce islandicin, though in very varying yields, whilst the sixth gives no islandicin but a mixture of pigments of which three have been identified as chrysophanic acid, skyrin and flavoskyrin. Apart from actual differences in metabolic products it is necessary to take into account the changes in pH of the substrate, caused by the activity of the fungus, and the effect this change has on many pigments.

It behoves taxonomists to exercise the greatest caution in using colour alone to separate species, or even to group them together.

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ACTION DE LA NEOPYRITHIAMINE, ANTIVITAMINE B₁, SUR LA CAROTINOGENESE DU *MUCOR HIEMALIS*. CONTRIBUTION A L'ETUDE DES INTERRELATIONS VITAMINIQUES

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La néopyrithiamine est une antivitamine B₁ très active. Elle bloque le développement des organismes hétérotrophes pour la thiamine en déplaçant cette dernière de son complexe actif. Nous avons montré qu'à part cet effet primaire, la néopyrithiamine agit profondément sur la biosynthèse et le métabolisme de dix vitamines hydrosolubles et qu'elle perturbe profondément l'équilibre vitaminique de l'organisme (*Phycomyces*, *Neurospora*, culture en milieu aseptique de racines de *Pisum*) (1).

Mucor hiemalis synthétise des quantités élevées de caroténoïdes constitués en la majeure partie de β -carotène (2). Cet organisme se prête aussi bien que *Phycomyces* à l'étude du mécanisme de la carotinogénèse et des inhibiteurs de cette dernière. *Mucor hiemalis* étant autotrophe pour la thiamine ne réagit que faiblement à la présence de la néopyrithiamine. Des expériences répétées et dont les résultats concordent attestent que l'antivitamine B₁ intervient dans la carotinogénèse. L'expérience suivante le démontre; les caroténoïdes sont dosés par spectrophotométrie.

néopyrithiamine, en m γ p. 25 cm ³ de milieu								
	0	0,5	1	10	50	100	150	200
poids culture en mg	90	86,5	89,8	91	89,8	80,8	74,6	71,5
Caroténoïdes synthétisés en γ par g. sec de thalle	101,8 (100)	131,1 (129)	147,2 (145)	165,9 (163)	109,6 (107,5)	100,3 (99)	98,3 (96,5)	97,3 (95,5)

On voit donc que de très faibles doses de néopyrithiamine, de l'ordre de 1 à 10 mγ, détermine une hypercarotinogénèse très nette qui s'atténue avec des doses plus élevées de l'antivitamine B₁. L'avitaminose B₁ partielle déterminée chez *Mucor hiemalis* par la néopyrithiamine s'exprime non seulement par une perturbation de la biosynthèse de diverses vitamines hydrosolubles du groupe B mais aussi par une modification de la carotinogénèse.

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2. — *DETERMINISME DE LA FORMATION
DES APPAREILS DE FRUCTIFICATION
CHEZ LES CHAMPIGNONS
(DANS LA NATURE ET EXPERIMENTALEMENT)*

RAPPORT

**CAUSES OF FORMATION OF FRUCTIFICATION ORGANS
OF FUNGI (IN NATURE AND EXPERIMENTALLY)**

by Lilian E. HAWKER

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Mycologists have always been interested in the factors influencing the production of spores by fungi. Biologically the change from the vegetative to the reproductive phase is important in any group of living organisms. In the fungi, spores not only reproduce the individual, but are often formed in such large numbers that, given favourable conditions, they also achieve multiplication and dispersal or spread of the species. They often carry new combinations of factors which may lead to the colonization of new types of habitat. Some have great powers of resistance to temporary conditions sufficiently unfavourable to prevent mycelial growth. Not only is the intrinsic interest of the process of sporulation considerable but the practical significance is equally important. The identification of fungi usually depends on spore characters and a knowledge of the factors controlling sporulation often leads to the intelligent control of parasitic or other destructive fungi.

Many fungi produce more than one type of spore. The asexual zoospores, sporangiospores or conidia are usually produced in relatively large numbers and serve to spread the fungus during conditions favourable to vegetative growth. In general the conditions favouring mycelial growth also favour the production of the asexual spores. The title of this symposium, however excludes a consideration of the factors influencing this type of sporulation and directs our attention to the so-called « perfect » stage, that is to spores produced either as a result of a true sexual fusion of nuclei or of a fusion between two associated nuclei not necessarily of opposite sex. This type of sporulation, or fructification, in contrast to the production of asexual spores, is often initiated only when conditions have become unfavourable for mycelial growth.

The early work of KLEBS (1896, 1898, 1899, 1900) laid the foundations of our knowledge of the factors influencing fruiting in fungi. He pointed out that living cells are influenced first by their structure, which is

hereditary, secondly by internal conditions and thirdly by external ones and he indicated a number of external factors influencing fruiting. KLEBS's work has stood the test of time and more than 50 years later we are still working within the framework of his observations although we are far from having filled in the picture as yet.

Internal factors. — Every mycologist who has grown a fungus in culture knows that fruiting does not begin immediately after inoculation but is delayed for a period varying with the species and with external conditions. It is clear that the internal conditions within the hyphae must reach a stage which may be termed « ripeness to spore » before fruiting is initiated. We have very little idea what this condition is but it is probably related to the synthesis by the cells of complex enzyme systems and the accumulation of reserves of energy-rich substances. Some fungi can achieve this with a minimum of external material, others must be provided with external supplies, either of the enzymes themselves or of complex precursors of these or of their co-enzymes. Again some can fruit under a wide range of physical conditions and others will only do so within precise and narrow limits. It is difficult or impossible to decide where the effect of internal conditions ends and that of external ones begins since the former are a complex resulting from heredity and environment. Nevertheless it is well known that even the most favourable combinations of external factors cannot reduce the interval between inoculation and the initiation of fruiting below a definite period characteristic of the individual fungus. Often autolysis of some of the older cells precedes fruiting and this release of complex substances may even be a pre-requisite with some species.

External factors. — Physical factors such as temperature, water content of the substrate, humidity of the air and aeration, all exert a profound influence on both growth and all types of sporulation.

In general the range of temperature which permits fruiting is narrower, often considerably narrower, than that allowing mycelial growth or even the production of asexual spores. The optimum for fruiting may differ from that for vegetative growth, as when, with many plant parasites, the onset of fruiting occurs in the autumn in association with falling temperature (although this common phenomenon admits of other interpretations). In culture the temperature optimal for fruiting may not be the same on all media. The limiting effect of temperature may come into operation at a particular stage of the fruiting process, as with certain species of Mucorales where low temperature (around 7°C) inhibits the early stages of conjugation but does not prevent the maturation of zygospores already initiated (unpublished work at Bristol). Fusion between sporidia of *Ustilago maydis* takes place freely only over the narrow temperature range of 20-24°C (BOWMAN 1946). Studies of this type may throw light on the nature of the processes contributing to fructification at different stages. Most fungi require

more moisture for sporulation than the minimum which permits mycelial growth. *Magnusia brachytricha* is said to grow over a range of 29.5-100 % relative humidity and to produce perithecia only over a ranges of 49-100 %. (SWEET, 1942). Precise studies on water requirements for fruiting are almost entirely lacking. It is known, however, that fruit-bodies of agarics subjected to a current of dry air may develop abnormally (KEYWORTH, 1942) suggesting that humidity is an important factor controlling normal development. It is obvious that the concentration of food materials which takes place in most fruit-bodies can only be achieved by the evaporation of much of the water flowing into these organs from the mycelium. Hence one would expect the range of humidity permitting the development of complex fruit-bodies to be narrower than that over which vegetative growth takes place.

Aeration is another important factor which has received too little attention. Again the evidence available suggests that the requirements for fruiting are more exacting than those for mycelial growth. e.g. DENNY's (1933) work with *Neurospora sitophila*.

It is well known that many Ascomycetes and Basidiomycetes require a short period of illumination before fruit-bodies are produced. While much empirical data on this subject has been accumulated no precise attempt to explain this need for light has been reported. One would expect light to act through the supply of energy resulting in the synthesis by the fungus of some essential enzyme or the activation of a preformed lightsensitive substance.

H-ion concentration of the substrate is another factor with a profound effect on growth and sporulation and again such evidence as we possess shows that fruiting is possible only over a narrower range than that permitting vegetative growth and asexual sporulation.

Mechanical barriers to growth, contact with hard surfaces and injury to the mycelium have all been claimed to stimulate fruiting in certain fungi but the mechanisms of these stimuli are entirely unknown.

The most important single factors determining the initiation and maturation of sexual spores or fruit-bodies is nutrition. This is to be expected when one considers the amount of complex substances, enzymes, food reserves etc. which are required during the process of fruiting.

The concentration of the various food substances, and particularly of carbohydrates or nitrogenous substances has a profound influence on the type of growth. The optimum concentration for mycelial growth is almost always too high for the initiation of fruiting (HAWKER 1936, 1939) but the concentration optimal for the production of fruit-body initials in culture may not permit their further development and maturation. This difficulty may be overcome either by growth on a rich medium and subsequent transfer of the well-fed mycelium to a more dilute medium (e.g. CLAUSSEN

1912), by the provision of a large amount of nutrients by increments so that the concentrates of the medium remains low while the total food substances supplied is relatively high (HAWKER 1939), or by growing the fungus in a large volume of dilute medium (PLUNKETT 1953). The *relative* concentrations of the various food substances in the medium is also important. The virtues of the so-called balanced medium are familiar to all experimental mycologists. The ratio of carbon to nitrogen, in particular, influences the type of growth, as with certain species of *Botrytis* (*Sclerotinia*) where a high concentration of nitrogen favours the production of conidia while sclerotia, which may be considered to be the initial stages in apothecial formation, are favoured by a high concentration of carbohydrate. (TOWNSEND 1952).

The nature of the available food substances is also of importance. Very little exact information exists as to the effect of minerals (either as macro- or micro- nutrients) on fruiting. Recently BASU (1951) has claimed that calcium is essential for fruiting of *Chaetomium globosum*.

There have been a number of claims that certain organic nitrogenous substances are superior to inorganic ones in their effect on fruiting of various fungi. While this is inherently likely yet it is difficult to separate the effect of chemical structure from that of traces of growth-substances present as impurities.

The effect of the nature of the carbon source has been studied in greater detail. In general it may be said that whereas, mycelial growth is favoured by hexose sugars, particularly glucose and fructose, fruiting often takes place more readily with a more complex carbohydrate. In a study of perithecial formation in *Sordaria* (*Melanospora*) *destruens* and in some other Pyrenomycetes (HAWKER 1936, HAWKER and CHAUDHURI 1946) I was able to show that the most favourable carbohydrates were those which the fungus was able to hydrolise at such a rate that a concentration of hexose sugar optimal for fruiting was maintained over a relatively long period. With *S. destruens* this was sucrose. Nevertheless this was not the whole story since the effect of sucrose could not be exactly reproduced by supplying the fungus with glucose and fructose in small doses. It was found that the greater ease of phosphorylation of sucrose was also an important factor. The addition of small amounts of fructose diphosphate or of glucose-1-phosphate to a glucose medium had a striking effect on fruiting (HAWKER 1947, 1948). More recently BUSTON and his co-workers (BUSTON and KING 1951) have shown that the favourable effect of an extract of jute on the formation of perithecia by *Chaetomium globosum* was also in part due to the presence of traces of phosphoric esters and have confirmed their results by the use of paper chromatography (BUSTON, JABBAR and ETHERIDGE 1953). Respiration of *S. destruens* was more rapid on a sucrose than on a glucose medium, suggesting that a relatively greater amount of energy

was released for use in the synthesis of the many complex substances required in fruiting (HAWKER 1947).

It is well known that many fungi are able to grow and sporulate on a simple synthetic medium but that others require an external supply of certain growth-substances or vitamins, notably of vitamin B₁ (aneurin or thiamin). The latter are said to be deficient for the particular growth-substance, which they are unable to synthesise or synthesise only in sub-optimal amounts. It is not surprising that the amount of such a growth substance required for fruiting is greater than that sufficing for vegetative growth. An external supply of thiamin increases fruiting in many fungi representing all the main groups, while a number require biotin. A strain of *S. destruens* (HAWKER 1939) is unable to grow on a glucose salts medium but could produce mycelium when biotin was added. The further addition of thiamin was necessary for the formation of perithecia (HAWKER 1939a). The amount of glucose optimal for fruiting increased with increase in concentration of thiamin and it was shown that this was associated with an increase in respiration rate (HAWKER 1942, 1944). Some vitamins have little influence on the number of fruit-bodies formed but are essential for the maturation of the spores. Thus BARNETT and LILLY (1947) showed that biotin starvation prevented the formation of viable ascospores by *Sordaria fimicola*. The same workers (LILLY and BARNETT 1947) showed that if a thiamin-starved mycelium of *Ceratostomella fimbriata* grown on an otherwise rich medium were transferred to distilled water no perithecia developed, but if thiamin were added to the water further perithecia were formed a few days after the transfer of the mycelium. This experiment supports the view that thiamin acts directly on the fruiting process and not indirectly through its effect on mycelial growth and vigour.

While thiamin, and perhaps biotin also, have a direct influence on fruiting they are not specific growth-substances. Recently a number of examples of substances of a hormone nature having an effect limited to a single species of fungus or to a particular stage in the formation of the fruit-bodies of that fungus, have been described. The most fully worked-out example is that of dioecious species of *Achlya* which RAPER (1939, 1940, 1942, 1950) has shown to produce antheridia and oogonia in response to the synthesis of a series of hormones by the male and female thalli respectively. The first of these, termed the hormone A group, is produced by the female vegetative hyphae and induces the formation of antheridial initials. Its exact nature is not yet known but it can be replaced to some extent by various organic acids or by hydantoin (RAPER and HAAGEN-SMIT 1942). The hormones which control the later stages in reproduction are difficult or impossible to obtain in quantity and thus their chemical nature is unknown.

Conjugation of species of *Zygosaccharomyces* (NICKERSON and THI-

MANN 1941, 1943) is stimulated by two substances which can be replaced by a mixture of glutaric acid and riboflavin while maturation of zygospores of *Phycomyces blakesleeanus* also requires the presence of specific growth factors. (ROBBINS and SCHMIDT 1945).

EMERSON and FOX (1940) found that γ -carotene was present in male cells of *Allomyces* but was absent from female or vegetative cells.

The course of events when two colonies of opposite strains of certain heterothallic Zygomycetes approach one another suggests that conjugation is controlled by the production of labile hormonal substances. BURNETT (1953) has recently shown that oxygen consumption rises rapidly before the hyphae of the opposing strains come into contact, thus supporting the theory that these strains produce complementary hormonal substances which must be of a labile nature since they have never been extracted or preserved in the absence of the living hyphae. The specific nature of such substances was confirmed by BURNETT's (1953a) discovery that a similar but less intense increase in oxygen consumption occurs when heterothallic and homothallic strains are paired in an attempt to produce imperfect hybridization. Failure to produce these hybrids is then attributed to slight differences in the hormones produced by different species. It is of interest that again reproduction is preceeded by an increase in rate of respiration.

While recent work points the way to some sort of understanding of the factors influencing reproduction in culture there is still much to be done. When we consider factors influencing fruiting in the natural condition, that is in the field, we have hardly made a beginning. Studies such as those of Wilkins and his co-workers (WILKINS and PATRICK 1940) suggest that each species of agaric responds to a particular combination of temperature and rainfall in the period preceeding sporophore formation. My own recent study, still in progress, of a number of hypogeous fungi, similarly indicates the importance of soil and weather conditions as factors controlling fructification and shows that species differ widely in their requirements (HAWKER 1954). We have a mass of detail, difficult to analyse, relating to sporulation of parasitic fungi. We have practically no data relating to nutrition in the field. The ecological approach to the study of fungi in the field has been neglected by most mycologists but is essential if we are to apply the findings of pure culture work to the infinitely more complex problem of fruiting under natural conditions.

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THE ROLE OF METABOLISM IN MORPHOLOGICAL DIFFERENTIATION OF THE WATER FUNGUS, *BLASTOCLADIELLA EMERSONII*

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Blastocladiella emersonii, a primitive, arbusculate, aquatic fungus with a determinate system of growth, liberates motile, unflagellate swimmers which are capable of developing into either of two mature thallus types. One bears and apical, thin-walled spore-sac with prominent discharge papillae, and the other bears a distinctly-different, apical, thick-walled, pitted, brown resistant sporangium (RS). This particular phase of morphogenesis can be controlled at will; in the presence of 10-2 M bicarbonate on agar media, R.S. plants are formed, whereas in its absence, thin walled plants are formed instead.

On the other hand, we have also obtained mutant strains of *B. emersonii* which are readily distinguished from the wild type by virtue of their content of gamma-carotene. Because they do not respond to the bicarbonate stimulus and are generally incapable of producing R.S. plants, the mode of development of these mutants is limited to but a single morphogenetic pathway.

During ontogenesis of R.S. plants, there exists a critical period beyond which the morphogenetic mechanism cannot be altered by decreasing the concentration of external bicarbonate. Apparently, this phenomenon is directly related to the occurrence of a progressive decrease in permeability of the thallus to bicarbonate during differentiation. Concomitantly, this imposes a gradually-increasing restriction upon the quantity of external bicarbonate that can penetrate the thallus membranes, as well as the quantity of metabolic bicarbonate, produced internally, which can escape to the outside. Thus, the metabolic reactions leading to the synthesis of chitin, fat, and melanin which are induced by increased concentrations of bicarbonate and which must, at least in part, be responsible for the genesis of a resistant sporangium, are assured continuity by a kind of internal « autocatalysis » until differentiation is complete.

Having established a quantitative relationship between the external stimulus (bicarbonate) and the morphogenetic response, attempts were made to elucidate the biological significance of the « trigger mechanism » in terms of the more fundamental and causal biochemical reactions involved. Indirect but extensive and generally consistent evidence, derived from

in vivo studies with the wild type, led to the conclusion that bicarbonate probably tended to decrease the rate of a biotin-dependent oxidation of alpha-ketoglutarate. Thus, it was believed that some degree of accumulation of intermediates between ketoglutarate and citrate, or its precursory C_2 compounds, was occurring within the fungus; this, in turn, somehow initiated the biosynthesis of melanin and other compounds associated with the genesis of a resistant sporangium. Since then, it has been shown that it is possible to uncouple at least one of these reaction chains without seriously interfering with either the overall developmental pattern or the basic structure and behavior of the final product. For example, the synthesis of melanin can be inhibited *in vivo* with phenylthiourea; under these conditions, bicarbonate induces the formation of structurally-normal and perfectly viable but absolutely colorless resistant sporangia which are apparently completely devoid of melanin.

More recently, direct, experimental evidence derived from *in vitro* studies of cell-free preparations have significantly strengthened these concepts regarding the function of bicarbonate in morphogenesis. First, with chromatographic and spectrophotometric techniques, it has been demonstrated that when living cells of *Blastocladiella* are placed in contact with bicarbonate for different time intervals and then quickly killed and homogenized, the concentration of keto acids found therein is greater than found in homogenates derived from untreated plants. Second, in current attempts to elucidate the nature of the coupling between the bicarbonate inhibition and the induction of melanin synthesis, it has become abundantly clear that polyphenol oxidase is always found in resistant sporangia, where it appears to be rather firmly bound to the chitinous wall; it cannot be detected in thin walled plants of various ages grown under a variety of environmental conditions. Finally, spectrophotometric studies with cell free preparations of the wild type *Blastocladiella* have revealed the presence therein of cytochrome oxidase, aconitase, TPN-specific isocitric dehydrogenase, DPN-dependent ketoglutarate oxidase, succinoxidase, fumarase, and DPN-dependent malic (dehydrogenase) activity. As in the *in vivo* studies, biotin again appeared to play a role in the oxidation of the ketoglutarate. It was discovered, however, that mutant strains which, unlike the wild type, are incapable of forming resistant sporangia in response to bicarbonate, do not possess aconitase and alpha-ketoglutarate oxidase (*the presumed locus of the bicarbonate effect!*) although all the other enzymes found in *B. emersonii* are present. Thus, the two, successive, oxidative decarboxylations in the sequence of reactions leading from citrate to succinate in *B. emersonii* are inoperative in the mutant. These observations offer strong, corroborative evidence for the postulations regarding the morphogenetic mechanism in *B. emersonii*.

In summary, the arbusculate thalli of *Blastocladiella* have a deter-

minate system of growth. Furthermore, on solid substrata in the absence of added bicarbonate, the growth of colonies derived therefrom ceases after several generations. Cessation of growth is presumably due, at least in part, to the accumulation of metabolic CO_2 (as bicarbonate) which induces most members in these densely crowded colonies to assume the resting stage (R.S.). Thus, growth of the colony is terminated because of the liberation of a metabolic product which initiates differentiation of its members into non-proliferating plants. On the other hand, whereas the arbusculate thalli of the mutant strains are also determinante in their system of growth, the colonies derived therefrom are not. The peripheral spread of such colonies does not cease but, instead, continues indefinitely so long as nutrient supply is available for new cell generations. The mutant has lost the enzymes (alpha-ketoglutarate oxidase and aconitase) which, in the normal strain, respond to the accumulation of metabolic CO_2 and thus impose a limitation on growth by bringing about differentiation; it continues, therefore, to proliferate indefinitely.

INFLUENCE OF CERTAIN ENVIRONMENTAL FACTORS ON THE GROWTH AND SPORULATION OF THE DERMATOPHYTES

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The growth rate of the Dermatophytes is highest at a temperature of about 30°C . The temperature most favourable for conidia development seems, however, to lie some degrees below that of the optimum for growth. The range for conidia development is wider below than above the optimum for sporulation. The number of the conidia mounts slowly in accordance with temperature rise above zero, but after having passed the maximum it sinks rapidly. Also the shape of the conidia, and especially that of the macroconidia, on which latter the botanical classifications have been based, follows, on the whole, the numeral occurrence of the structures. At favourable temperature ranges the shape of the macroconidia is classical or normal. But it is less differentiated or becomes retrogressive in unfavourable conditions. Closer to the temperature limits for growth the retrogression process in cultures may go even further, the cultures becoming deprived of aerial mycelium at all. The colonies then turn glabrous, and the only structures developed are chlamydospores. The

retrogressive forms of macroconidia have often been interpreted as independent form-types and have thus served as bases for creating of new, but actually non-valid species or genera.. The form changes of the macroconidia as well as transformations of the colony growth type can be effectuated also by other physico-chemical factors, inter alia, by toxic compounds, such as fungicides or fungistatics.

THE AERATION COMPLEX OF FACTORS AND FRUIT-BODY FORMATION IN PURE CULTURES OF HYMENOMYCETES

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The production of hymenomycete fruit bodies in pure culture is now commonplace. Seldom, however, are they normal. The internal atmosphere of a culture vessel often has a high humidity; CO_2 and other volatile metabolic products may accumulate and O_2 tension may be reduced. Controlled experiments on the possible influence of these factors are almost lacking although it is known that « aeration » may be beneficial.

In experiments with *Collybia velutipes* (Curt. ex Fr.) Quél. sterile gas mixtures of known composition and controlled and measured relative humidity have been passed at known rates over pure cultures in sterile chambers. The level of the liquid culture media has been maintained automatically from reservoirs of sterile distilled water. Temperature and illumination have been controlled.

In unrenewed air stipes of *Collybia velutipes* lacking all but the minutest pileus rudiments develop, whereas in parallel cultures over 5 % KOH or with moving air normal pilei form over a wide range of humidities. Preliminary trials, the results of which are only accepted tentatively, showed that gas streams similar to air but with CO_2 enriched at the expense of N_2 , caused an inhibition of pileus development. This was evident with 1 % CO_2 and at 5 % CO_2 stipe development too was arrested at an early stage. LAKON's dramatic conclusion (1908) that aeration substitutes for the morphogenic effect of light on pileus development (in a coprophilous *Coprinus* sp.) was inapplicable, as *C. velutipes* required both light and aeration (? removal of CO_2) for this process.

In illuminated cultures at 25 — 30° C, *Polyporellus brumalis* (Pers.) Karsten makes maximum growth but does not fructify. Usually epileate

stipes, however, are produced at 20° C in this species and in *Polystictus perennis* (L.) Fr.. Various light intensities and durations and uv irradiation failed to induce pileus formation. At about 15° C and below stipes bearing fertile pilei occur. Such caps are more less abnormal whether the culture vessels are sealed or have loosely fitting lids. The closest approach to normality occurred in apparently fairly dry vessels. At the time of writing experiments with controlled air streams at 15° C are being set up.

The absence of normal pilei is perhaps the most usual feature of recorded sporophore freaks in culture. Both my own work and a survey of the literature suggests that pileus formation may prove to be a critical stage in fructification highly sensitive to environmental factors. MAGNUS's (1606) views on the correlation of stipe and pileus growth, if substantiated, might indicate that such effects as excessive elongation (and branching) of the stipe are only secondary and due to the absence of a restraint imposed by the pileus.

3. — ACTION DES FACTEURS DE CROISSANCE SUR LES CHAMPIGNONS

RAPPORT

LES FACTEURS DE CROISSANCE POUR LES MICROORGANISMES

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Peu de problèmes se sont développés, au cours des vingt dernières années, d'une manière aussi extraordinaire que celui des facteurs de croissance pour les microorganismes. Des milliers de travaux spéciaux, des revues d'ensemble, des livres ont paru. Il n'est donc pas question de donner un résumé, même restreint, de la question. Nous nous bornerons à évoquer les faits principaux sur lesquels sont basées nos conceptions actuelles en nous limitant aux citations essentielles, et à esquisser le cadre dans lequel nos discussions auront lieu.

Le problème se pose entre 1860 et 1870, à la suite d'une controverse entre Pasteur et Liebig qui, les deux, voulaient cultiver la Levure sur un milieu synthétique. Les recherches d'E. WILDIERS (1901) déterminent un premier progrès. Ce savant démontre que la culture de la Levure en milieu synthétique n'est possible qu'en présence d'un facteur inconnu qu'il appelle « Bios ».

Dès 1900 la vitaminologie se développe à la suite des découvertes d'EIJKMANN relatives au facteur antipolynévritique (vitamine B₁); l'étude des facteurs de croissance pour les microorganismes en profitera largement. On découvre la nature multiple du bios. En 1928, Miss EASTCOTT reconnaît que le méso-inositol est l'un des facteurs de croissance pour la Levure. On ne comprit pas immédiatement l'importance de cette découverte, le rôle du Méso-inositol étant inconnu. En 1934 (SCHOPFER), il est démontré que la vitamine B₁ (aneurine, thiamine) est le facteur de croissance nécessaire pour *Phycomyces blakesleeanus* en milieu synthétique. Pour la première fois, on constate qu'une vitamine dite « animale » est un facteur de croissance pour un microorganisme. A. LWOFF (1933-34) montre que la protohémine est facteur de croissance pour *Strigomonas fasciculata*. KNIGHT (1935-1937) reconnaît que l'acide nicotinique est facteur de croissance pour *Staphylococcus aureus*. Enfin, en 1936, KOEGL isole la biotine (vitamine H) constituant essentiel de l'ancien bios.

Dès ce moment, les découvertes se multiplient. L'observation qu'une

vitamine, telle que la thiamine, est un facteur de croissance établi entre les physiologies animale et végétale une liaison dont on verra qu'elle a été féconde en résultats. En effet, les principales vitamines se révèlent être des facteurs de croissance pour les microorganismes : thiamine pour *Phycomyces*, de nombreux Champignons et Bactéries, riboflavine pour *Lactobacillus casei*, pyridoxine (vitamine B₆) pour *Ceratostomella ulmi*, biotine (vitamine H) pour diverses Levures, Champignons et Bactéries, acide pantothénique, pour *Lactobacillus arabinosus*, le méso-inositol pour diverses Levures et Champignons, l'acide folique pour *Streptococcus faecalis*, l'acide nicotinique et la nicotinamide (vitamine PP) pour diverses Bactéries, la vitamine B₁₂ pour *Euglena gracilis* var. *bacillaris*; le cholestérol est facteur de croissance pour *Trichomonas columbae*, l'acide pimélique pour *Corynebacterium diphtheriae* etc., etc.

L'action du facteur de croissance est :

1°) *quantitative*, d'où possibilité d'utiliser un microorganisme pour la détermination quantitative d'une vitamine ou d'un acide aminé. Les observations tendant à prouver qu'une très petite quantité de vitamine peut, par catalyse, déclencher un pouvoir de synthèse libérant l'organisme d'un apport extérieur de facteur de croissance sont des plus intéressantes, mais sujettes à discussion quant à leur interprétation.

2°) *Spécifique*; une faible modification de la structure de la molécule détermine une diminution de son activité, ou son inactivation.

3°) *Liée à la composition du milieu*.

Le facteur de croissance est essentiel lorsqu'en son absence l'organisme ne se multiplie pas; il est complémentaire lorsque, sans être indispensable, il détermine une intensification de la multiplication.

Le besoin en facteur de croissance est déterminé par une *perte de pouvoir de synthèse*. Le microorganisme réagit comme la cellule animale auxo-hétérotrophe. Il est intéressant de relever que C. FUNK, dans son Traité de vitaminologie (1924), avait compris que les plantes, en général, sont douées du pouvoir de synthétiser les vitamines, ce qui n'est pas le cas pour l'animal. Cette distinction, très schématique et correspondant à l'état de la science d'alors doit être aujourd'hui exprimée d'une manière plus nuancée. Quoi qu'il en soit, FUNK avait vu que toutes les plantes et tous les organes d'une plante ne *manifestaient* pas les mêmes pouvoirs de synthèse et compris la direction dans laquelle les recherches devaient se développer; les résultats obtenus depuis 20 ans confirment l'exactitude de ses vues.

Les pertes de pouvoir de synthèse peuvent, dans la limite des possibilités expérimentales, être *irréversibles*. On ne peut être certain de cette irréversibilité que si l'on a épuisé toutes les possibilités culturales applicables à l'organisme étudié.

Des observations anciennes ont montré qu'une modification de la com-

déshydrase), riboflavine (diaphorase I et II), etc. La qualité de co-facteur qu'assume la vitamine explique toutes les caractéristiques du facteur de croissance : spécificité d'action, action à faible dose, etc.

Le problème du remplacement des facteurs s'explique en partie par le fait que le produit du métabolisme formé grâce au co-facteur dont la vitamine est le constituant actif, peut remplacer cette dernière : désoxyriboside de la thymine, de la guanine, de l'hypoxanthine remplaçant la vitamine B₁₂ pour *Lbc. leichmannii* et pour *Euglena gracilis* var. *bacillaris*.

Un phénomène de remplacement d'un autre type peut se produire lorsque, dans une constellation, l'élévation du taux de l'une des vitamines du groupe compense la diminution du taux d'une autre vitamine.

L'attention s'est portée tout d'abord sur les pertes de pouvoir de synthèse déterminant des besoins en facteurs de croissance. Ces recherches ont conduit naturellement à étudier le problème sous un autre aspect, celui des biosynthèses et de leurs mécanismes. L'auxo-hétérotrophie et l'auxo-autotrophie doivent être l'une et l'autre l'objet de recherches si l'on veut envisager le problème dans son ensemble. Les premiers travaux relatifs au fractionnement de l'hétérotrophie ont ouvert la voie et nous ont montré qu'une molécule de vitamine se forme par étapes. Cependant, les données restent très fragmentaires et tous les moyens sont mis en œuvre pour comprendre le mécanisme d'une biosynthèse chez un organisme auxo-autotrophe.

La méthode des éléments marqués peut rendre d'éminents services. Un exemple le démontre ; il peut être démontré qu'en utilisant de l'acétate marqué (¹⁴C dans le CH₃ et le COOH) offert à *Phycomyces*, le 69 % des atomes de C du β-carotène synthétisé par cet organisme provient de l'acétate. Le corps en C₂ est donc, dans des conditions bien définies, le précurseur du corps en C₄₀ (GROB et SCHOPFER).

Bien des problèmes restent à élucider. Nous en citons un qui retient actuellement l'attention : l'intervention des éléments traces (éléments oligo-synergiques) dans la biosynthèse d'une vitamine. Si l'on ne prend pas des précautions spéciales, ces éléments sont présents dans tous les milieux de culture. LAVOLLAY et LABORAY ont, les premiers, montré qu'*Aspergillus niger* forme abondamment de la riboflavine lorsque l'organisme se trouve en carence de Mg. Dans des conditions culturales telles que l'intervention des éléments traces peut être supposée, *Phycomyces* devient partiellement autotrophe pour la vitamine B₁ (SCHOPFER, 1953). Il n'est pas impossible que des éléments oligosynergiques en excès paralysent une bio-synthèse et déterminent une auxo-hétérotrophie qui peut être rendue réversible en modifiant la composition du milieu.

Les organismes auxo-hétérotrophes, malgré les découvertes qui s'accumulent, sont en minorité par rapport aux organismes auxo-autotrophes. La supposition qu'ils naissent par mutation avait déjà été émise, sans que des expériences décisives justifient l'exactitude de ce point de vue. Dès 1941, les travaux de BEADLE et de TATUM et de l'école américaine ont renouvelé complètement le problème en le plaçant sur le plan de la génétique. Ces travaux portent sur *Neurospora crassa* et *Neurospora sitophila*, Ascomycètes hétérothalliques faciles à cultiver. L'irradiation des conidies (rayons UV, X), ou leur traitement par des substances mutagènes, déterminent la production de mutations physiologiques caractérisées par la perte d'un pouvoir de synthèse affectant une vitamine, thiamine ou ses constituants, riboflavine, pyridoxine, acide pantothénique, acide nicotinique, acide p-aminobenzoïque, des acides aminés, etc. Des mutations portant sur des purines ont été également signalées par FRIES, chez *Ceratostomella multiannulata*.

Les mutations de *Neurospora* sont héréditaires. Leur transmission satisfait aux lois de Mendel. *Neurospora* est un haplobionte; la répartition des caractères dans la première génération est celle que la génétique des haplontes permet d'attendre; le croisement d'une forme sauvage et d'une forme « pantothenicless », devenue hétérotrophe pour l'acide pantothénique, produit 50 % de chacune des deux formes. Le pouvoir de synthèse est donc contrôlé par un gène qui, par une série de réactions dans lesquelles le cytoplasme est impliqué, conduit à la biosynthèse de la vitamine considérée.

Il a été possible de trouver, partiellement du moins, et pour certaines vitamines, la chaîne de réaction, dont chaque étape est contrôlée génétiquement, et conduisant à la biosynthèse du facteur de croissance. C'est le cas, par exemple, pour l'acide nicotinique qui se forme par les étapes suivantes: acide anthranilique → indole → tryptophane → acide cynurénique → acide oxycynurénique → acide 3-oxy-anthranilique → acide nicotinique → cozymase (BEADLE, MITCHELL et NYC; MITCHELL et NYC).

En ce qui concerne la thiamine, les mutants expérimentaux obtenus sont identiques à ceux, naturels, connus auparavant; un *Neurospora* a été créé qui, comme *Phycomyces*, sait utiliser la pyrimidine et le thiazol au lieu de thiamine.

Les recherches que nous venons d'évoquer ont déterminé des progrès considérables dans nos connaissances relatives à la biosynthèse de certaines vitamines et de quelques acides aminés.

Fait singulier, ces mutations peuvent être rendues réversibles. Un *Neurospora crassa* « leucineless » devient indépendant s'il est cultivé en présence de doses définies de leucine. RYAN, qui a étudié ce phénomène, nous en donne une explication.

Ces mutations « en retour » peuvent également être déclanchées par des agents physiques et chimiques. WESTERGAARD et KOELMARK font à ce sujet des observations fort intéressantes. Un *Neurospora crassa* « adeninless »

réagit nettement à la présence d'agents mutagènes tels que l'oxyde d'éthylène, le diépoxybutane, le diépoxyde de l'éther propylique, l'éthylèneimine. Le diépoxybutane produit plus de mutations en retour que le traitement par les rayons UV et X et que le « mustard-gas ». Les souches deviennent autotrophes pour l'adénine.

GILES fait une étude spéciale du *Neurospora crassa* « inositolless ». Il utilise 14 souches qui sont identiques du point de vue physiologique et génétique. Elles diffèrent cependant par leur aptitude à fournir des mutations en retour après action de rayons UV ou de substances mutagènes. WESTERGAARD relève qu'un groupe d'agents mutagènes (Radiations, mustard nitrogen, peroxyde d'hydrogène, peroxydes organiques, formaldéhyde, diazométhane) est actif sur *Drosophila*, sur les chromosomes végétaux, sur les Bactéries, et sur *Neurospora* (mutations en retour).

Ces agents doivent déterminer la production de mutations géniques et de mutations chromosomiques. Ces recherches, les plus récentes dans le domaine des facteurs de croissance, nous permettent de faire diverses hypothèses au sujet du mécanisme d'action du facteur actif sur ce que l'on convient d'appeler un gène, ou plus simplement sur le mécanisme d'action de ce dernier.

Les avitaminoses déterminées par les divers agents évoqués peuvent être considérées comme *génotypiques*; le génome a été touché et c'est à son origine que le mécanisme de la biosynthèse a été troublé.

Un autre type d'avitaminoses, *phénotypiques*, peut être produit à l'aide d'*antivitamines*. Une antivitamine est une substance de constitution et de configuration très voisines de la vitamine correspondante; elle déplace cette dernière de son support; les réactions du métabolisme qui se déroulent grâce à la vitamine sont interrompues en un point déterminé et des produits intermédiaires s'accumulent. Nous disposons donc d'une autre méthode permettant de connaître les étapes d'une biosynthèse. L'antivitamine peut être déplacée à son tour par la vitamine correspondante et le phénomène est rendu réversible.

Les principales vitamines connues ont leur antivitamine spécifique: la pyrithiamine pour la thiamine, la dichloro-riboflavine pour la riboflavine, la pantothyltaurine pour l'acide pantothénique, les sulfamides pour l'acide p-aminobenzoïque. Le couple sulfamidé-APAB a été le premier étudié et se trouve à la base de la théorie des antivitaminés.

L'importance de la méthode est illustrée par l'exemple suivant. Des cultures d'*E. coli* inhibées par un sulfamidé peuvent être désinhibées par l'adénine (SHIVE et ROBERTS 1946). Il en est de même pour *Eremothecium Ashbyii* (SCHOPFER). Dans les cultures d'*E. coli* une substance s'accumule qui n'est autre que l'amide de l'acide 4-amino-imidazolcarbonique. Cette substance est le précurseur de la purine. L'acide p-aminobenzoïque, constituant de l'acide folique, fait partie d'un système enzymatique permettant

la biosynthèse des purines. Déplacé par le sulfamidé, l'APAB ne peut jouer son rôle et le précurseur de la purine s'accumule dans le milieu; cet arrêt d'une biosynthèse essentielle est la cause principale de l'inhibition déterminée par le sulfamidé.

L'action de l'antivitamine ne se limite pas, comme on le croyait, au déplacement ou à l'inactivation de la vitamine correspondante. L'exemple suivant le démontre.

La pyriéthamine est l'antivitamine spécifique de la thiamine (vitamine B₁). A très faible dose elle bloque le développement de *Neurospora crassa* « thiaminless »; l'inhibition est rendue réversible par la thiamine. Or, en effectuant une analyse complète des thalles et des milieux des cultures partiellement inhibées nous relevons les faits suivants: très forte hypervitaminose pour la biotine et pour la pyridoxine, hypervitaminose nette pour la riboflavine, l'acide pantothénique et l'acide nicotinique, accélération de la destruction, ou de l'inactivation de la thiamine, hypovitaminose pour l'acide folique.

On voit donc que, sous l'influence de l'antivitamine B₁, tout le système vitaminique de l'organisme est troublé; les biosynthèses ne s'accomplissent plus normalement. L'organisme est un tout tant physiologiquement que biochimiquement; une perturbation en un point se répercute dans tout le métabolisme.

On voit la variété des problèmes dans lesquels les facteurs de croissance sont impliqués. Evoquons encore quelques questions importantes. Les *facteurs de sexualité* et de reproduction nous semblent différents des facteurs de croissance ordinaires. Ils gouvernent d'une manière spécifique des phénomènes de morphogénèse; leur caractère hormonal est plus marqué que leur caractère vitaminique. Nous pensons aux travaux de RAPER sur *Achlya* et à ceux de CANTINO sur *Blastocladiella*. Il y a encore beaucoup à attendre de telles recherches.

Le problème si important de la *symbiose* a également été abordé du point de vue des vitamines. Des symbioses artificielles ont été créées en cultivant ensemble des champignons dont les besoins en vitamines sont complémentaires; *Mucor Ramannianus* réclamant le thiazol de l'aneurine, et *Rhodotorula rubra* la pyrimidine, peuvent être cultivés en culture mixte, sans facteurs de croissance, chacun des partenaires livrant à l'autre la moitié de la molécule nécessaire pour la synthèse de la molécule complète de thiamine. De nombreux faits de stimulation unilatérale ou bilatérale peuvent être expliqués de cette manière.

Le problème des facteurs de croissance est né du besoin de cultiver un microorganisme en milieu synthétique. Au cours des 20 dernières années il est devenu un problème de physiologie générale. Les facteurs vitaminiques requis par les microorganismes sont des co-ferments. Bactéries et Champignons ont ainsi permis de découvrir de nombreux faits nouveaux dans le

domaine de l'enzymologie; nous pensons aux travaux relatifs au coenzyme A (Lipman et son école), à l'acide lipoïque, facteur remplaçant l'acétate pour *Lbc. casei* et à la lipothiamide active dans la décarboxylation oxydative du pyruvate.

Enfin, le problème des facteurs de croissance nous a conduits dans le domaine de la génétique physiologique aux progrès de laquelle il a pris une part déterminante.

Nous ne citons ici que quelques ouvrages généraux donnant une bibliographie, partielle ou complète, de la question. Nous renonçons à citer dans cet exposé introductif les nombreux travaux spéciaux auxquels nous avons fait allusion.

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CROISSANCE, MORPHOGENESE, SYNERGISME ET ANTAGONISME NUTRITIFS CHEZ UN *PHYTOPHTHORA*

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Dans la croissance des champignons en milieux nutritifs solides *in vitro*, on peut distinguer l'élongation des hyphes que l'on mesure en prenant le diamètre des colonies et la ramification de ces hyphes qui rend l'épaisseur des colonies plus ou moins grande. Nous mesurons l'épaisseur des colonies par la lumière qu'elles interceptent quand elles sont interposées entre une source lumineuse et une cellule photoélectrique.

La ramification elle-même paraît pouvoir se décomposer en division nucléaire, — ou toute autre condition antérieure à la ramification proprement dite et nécessaire pour qu'elle ait lieu, — et l'allongement des ramifications hyphales. Chacune de ces « composantes » de la croissance exige la synthèse de substances plastiques spécifiques des hyphes. L'élongation, par exemple, se fait grâce à la synthèse des glucides qui entrent dans la composition de la paroi des hyphes, la division nucléaire exige la synthèse des désoxyribonucléoprotéines, etc.

Ces synthèses se réalisent grâce à la présence de substances ou facteurs de croissance qui sont eux-mêmes synthétisés par le champignon quand il est auxoautotrophe, mais qui doivent être ajoutés au milieu de culture quand il est hétérotrophe pour ces substances.

La forme ou « habitus » que le champignon prend dans les différents milieux de culture dépend de la stimulation différente que ces milieux exercent sur les composantes de la croissance.

Nous avons montré que chez un *Phytophthora* sp., l'aneurine, — un facteur de croissance que cet organisme ne peut pas synthétiser, — est indispensable pour assurer la ramification des hyphes, alors qu'elle n'exerce aucune influence sur le diamètre des colonies. Un facteur de croissance encore indéterminé chimiquement, que nous désignons par facteur L et qui existe dans les milieux de culture naturels et dans la gélose japonaise (*Gelidium*) mais pas dans certaines autres géloses, est nécessaire pour l'élongation des hyphes. Il doit être ajouté au milieu de culture (sous forme d'un extrait aqueux de gélose, par exemple) pour obtenir le développement maximum de *Phytophthora* en milieu synthétique liquide.

Les résultats suivants sont ceux d'une expérience, — parmi plusieurs où nous avons étudié l'effet de ces facteurs de croissance sur le poids du mycélium du *Phytophthora citrophthora*, — dans laquelle le champignon

a été cultivé pendant 17 jours, à 27°C, dans 25 cc de milieu de Czapek, additionné de la solution de Robbins qui contient les sels des ions métalliques qui jouent un rôle important dans la nutrition des champignons : Czapek seul, 10,4 mg. (poids sec du mycélium, moyenne de 5 résultats); le même milieu avec aneurine (4×10^{-2}), 78,4 mg.; avec extrait aqueux de gélose japonaise, 68,8 mg.; avec aneurine plus extrait, 2.099,4 mg. On voit que le facteur L contenu dans la gélose et l'aneurine produisent, quand ils sont associés, un effet synergétique considérable. Nous attribuons ce synergisme au fait que ces deux facteurs de croissance agissent sur deux composantes différentes de la croissance, l'élongation des hyphes et leur ramification.

Au contraire, l'asparagine qui n'est pas une substance de croissance indispensable pour le *Phytophthora* mais qui augmente la ramification, ajoute simplement son effet à celui de l'aneurine. Seule (à 1 %) dans le milieu de Czapek, elle a produit 69,2 mg.; avec l'aneurine, 138,2; avec l'extrait de gélose, 175,6. Les trois substances ensemble ont donné 3.168,2 mg. Il y a donc un faible synergisme de l'asparagine et du facteur L.

Les substances antagonistes de la croissance montrent des phénomènes comparables avec le *Phytophthora*. L'ion cuivre par exemple inhibe la croissance en diamètre sans affecter la ramification. Au contraire, l'ion nickel agit surtout sur la ramification. L'association de ces deux ions montre un effet de synergisme très marqué, l'inhibition de croissance étant largement supérieure à la somme de celles que ces ions exercent séparément.

Ces résultats ainsi que la conception du synergisme comme effet d'actions différentes sur les composantes de la croissance fournissent une base rationnelle pour l'étude des combinaisons de fongicides. Il a été jusqu'à présent fait fort peu usage des propriétés synergétiques des fongicides. L'association de fongicides qui effectuent l'inhibition de composantes différentes de la croissance devra permettre l'augmentation des propriétés fongicides sans accroissement des dosages ou, inversement, la réduction de ces derniers sans une diminution correspondante de leur efficacité.

REMARQUES SUR LES BESOINS EN FACTEURS DE CROISSANCE DES DERMATOPHYTES

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Le besoin en facteurs de croissance des dermatophytes a été l'objet de recherches de la part de divers auteurs, comme Schopfer, Robbins, Mackinnon, Georg, Benham et du fait de l'extrême variabilité des dermatophytes

cette étude doit être étendue à un grand nombre de souches de la même espèce.

De nombreuses souches de dermatophytes provenant de la collection de l'Institut Pasteur et de celle du Dr Rivalier de l'Hôpital Saint-Louis, ont été étudiées au cours de ces dernières années en ce qui concerne leurs besoins en facteurs de croissance vitaminiques ou acides aminés.

Plusieurs souches de *Trichophyton tonsurans* (crateriforme), *T. sabouraudi* (acuminatum), *T. sulfureum*, *T. violaceum* et son variant non pigmenté *T. glabrum* exigent la thiamine et en particulier la fraction pyrimidine, comme facteur de croissance dans un milieu de base contenant de l'azote ammoniacal ou aminé. Divers composés pyrimidiques autres que la pyrimidine de la thiamine sont incapables de remplacer cette dernière. Les souches de *T. faviforme* étudiées exigent la thiamine et l'inositol comme facteurs de croissance. *T. concentricum* est stimulé par la fraction thiazole. L'action de différents dérivés et précurseurs de l'acide nicotinique a été étudiée sur une souche de *T. equinum* (souche Baudet) que GEORG avait montré déficiente en acide nicotinique. L'hydrazide de l'acide nicotinique, l'acide 3-hydroxy-anthranilique, la cynurénine et, partiellement, le tryptophane peuvent remplacer comme facteur de croissance l'acide nicotinique ou son amide. L'acide anthranilique, l'acide cynurénique et l'acide indole- β -acétique ne peuvent remplacer ce facteur.

Les seuls dermatophytes exigeant un acide aminé sont ceux du groupe *T. rosaceum* (megnini) pour lesquels l'histidine est facteur essentiel de croissance. La carnosine (β alanyl-histidine) et l'histidyl-histidine peuvent remplacer l'histidine.

Les organes de reproduction asexuée rarement ou jamais observés sur milieux complexes chez certaines espèces de dermatophytes sont obtenus à partir de doses optimales de vitamines sur des milieux contenant une source favorable d'azote aminé.

Toutes les autres espèces de dermatophytes se sont montrées autotrophes, toutefois de nombreux facteurs complémentaires nécessaires à une croissance maximum et une morphogénèse complète restent à déterminer.

PURINES AS GROWTH FACTORS FOR FUNGI

by Nils FRIES

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Among the fungi no case is known where a purine or another nucleic acid constituent is normally needed as an indispensable component of the nutrient medium. Thus, no complete deficiency as to such factors seems to

occur. In some species, however, a growth-promoting effect of purines has been observed, which indicates a partial deficiency in the biosynthesis of these substances. As regards adenine, the strongest response has been found in species of *Mycena*, where the amount of dry matter produced after a certain period of time could be about ten times greater with, than without, this purine. Some wood-destroying polypores responded in the same way, although less strikingly. Guanine and hypoxanthine were active in some species, in others not.

Instead of, or together with, the positive effect on growth rate, adenine sometimes also caused an increase in the final amount of mycelium produced. All four possible modes of reaction were established: a) neither growth rate nor production affected (e.g. *Peniophora fraxinea*); b) only growth rate increased (e.g. *Mycena flavoalba*); c) only production increased (e.g. *Lentinus lepideus*); d) growth rate and production increased (e.g. *Polyporus annosus*). It may be questioned whether the signification of adenine as a growth factor in case c) is justified according to the usual implication of this concept.

The influence of adenine on the final amount of mycelium can perhaps be correlated with the essential role played by adenine in energy-transforming systems, a lack of adenine causing a less efficient coupling between oxydizing and synthesizing processes in the cells.

STUDIES IN THE GROWTH REQUIREMENTS OF INDIAN FUNGI

by T. S. SADASIVAN & C. V. SUBRAMANIAN

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Several fungi were tested by K. T. MATHEW and S. SURYANARAYANAN for their vitamin requirements. Out of 26 fungi tested the following were distinctly heterotrophic to thiamine hydrochloride: *Sclerotium oryzae* (rice); *Piricularia oryzae* (rice); *P. setariae* (*Setaria italica*); *P. zingiberi* (*Zingiber*); *Piricularia* spp. (3 isolates from *Eleusine coracana*, wheat and water grass); *Ceratostomella* sp. (*Cocos nucifera*); *Phytophthora arecae* (*Areca catechu*); *Pellicularia koleroga* (Coffee); *Corticium microsclerotia* (obtained from Baarn).

Detailed work on eight of the thiamine heterotrophic fungi listed above indicated that:

1. *Corticium microsclerotia* needed 2.0 μ g. of thiamine per 20/ml. of medium for optimum growth. The capacity to synthesize this vitamin, ho-

wever, was not entirely lost as the fungus did so from a mixture of thiazole and pyrimidine or from pyrimidine alone.

II. *Pellicularia koleroga* (including several morphologically similar isolates) also like *C. microsclerotia* responded to pyrimidine and thiazole together and, indeed, many isolates also responded to pyrimidine and thiazole separately as well as to riboflavine, biotin and pantothenic acid. However, varying degrees of response to these substances were noticed between isolates. On repeated subculturing in artificial media all isolates developed greater dependence on thiamine. Addition of other growth substances to media containing optimum amounts of thiamine had no effect except casein hydrolysate which increased growth considerably. Excess thiamine and biotin had no adverse effect on growth but the reverse took place with riboflavine.

III. *Piricularia oryzae*, *P. setariae*, *P. zingiberi* and *Sclerotium oryzae* were heterotrophic to thiamine. *P. oryzae* was unable to utilise either thiazole or pyrimidine during early growth but an equimolar mixture of both had thiamine replacement value with advancing age of the culture and also in the old culture pyrimidine was utilised by the fungus. *P. setariae* and *P. zingiberi*, however, needed both thiazole and pyrimidine; *Sclerotium oryzae*, on the other hand, grew with pyrimidine. Thus, *P. oryzae* (in young culture), *P. setariae* and *P. zingiberi* resemble *Phycomyces blakesleeanus* in the power to combine the thiamine moieties, *P. oryzae* in older cultures can complete thiamine synthesis from pyrimidine like *Sclerotium oryzae*.

IV. All species of *Piricularia* and isolates from *Eleusine coracana*, *Triticum vulgare* and *Brachiaria* are all biotin deficient, the deficiency being complementary. *Sclerotium oryzae*, on the other hand, is autotrophic to biotin. These results are in agreement with those of LEAVER but not of OTANI.

Similar physiological work done here on another aspect of growth requirement by fungi by (Miss) L. SARASWATHI DEVI on the heavy metal requirement of *Aspergillus niger* (standard « M » strain of Mulder) and an Indian isolate (designated *A. niger* strain « Mubl I ») indicated greater sensitivity to Co and Mo and less sensitivity to Mn for the Mubl I strain. Similarity in their responses to Fe- and Zn- deficiencies was, however, seen between the two strains although the Mubl I strain showed slightly increased sensitivity. These results indicate that in all probability the new strain either does not depend on a supply of Mo for growth and sporulation or it is able to utilise to the best advantage minute traces of this element left over in the purified medium, despite rigorous methods of purification employed, in which case it indicates a greater sensitivity to Mo detection than the standard « M » strain. Similar promising results useful in bioassay of Cu were obtained with the new strain.

COMMUNICATIONS DIVERSES

LES PRODUITS SECONDAIRES
DE LA FERMENTATION ALCOOLIQUE
DES DIVERS GENRES DE LEVURES

par Emile PEYNAUD, Madeleine LAFON, Jean RIBEREAU-GAYON

Faculté des Sciences de Bordeaux

La fermentation alcoolique anaérobie du sucre (glucose et lévulose) de moûts de raisin stériles, par une centaine de souches de *Saccharomyces ellipsoideus*, *Kloeckera apiculata*, *K. magna*, *Hanseniaspora Apuliensis*, *H. Guilliermondii*, *Saccharomyces Ludwigii*, *S. bisporus*, *Zygosaccharomyces polymorphus*, *Torulaspora Roseei*, n'a révélé aucune différence systématique dans le mode de fermentation du sucre par les divers genres examinés. Les dosages ont porté sur glycérol g, (molécules/litres), acide acétique a, succinique s, butylèneglycol b, acétylméthylcarbinol m, éthanal h. Dans tous les cas, se vérifie l'équation

$$5 s + 2 a + 2 m + b + h = g$$

avec une approximation de 10 % en général, g étant toujours plus grand que la somme indiquée.

A l'intérieur de l'espèce *S. ellipsoideus* existent, comme nous l'avons montré dès 1947, des races acétogènes, succinogènes, butylèneglycologènes ou peu butylèneglycologènes.

Un *Saccharomyces* de Geisenheim a donné, sur un jus de raisin désulfité, stérilisé, à 16° baumé, un vin à 8°,1, avec a/s=1, b/g=0,02 : c'est un type de levure succinogène et peu glycologène; un *Saccharomyces* de vin de Tokay donne a/s=4,3, b/g=0,1, type de levure acétogène et glycologène; ce même type de *Saccharomyces* a été retrouvé dans des souches d'Australie, du Portugal.

Un *Kloeckera apiculata* de Geisenheim a donné a/s=1,2, b/g=0,025, une *K. magna* des Pouilles a donné a/s=2,3, b/g=0,05; ces deux levures sont, l'une succinogène, l'autre acétogène, toutes deux peu glycologènes. Les *Saccharomyces*, qui donnent des vins à 8° (*S. Ludwigii*), ou à 10°,6 (*S. bisporus*) donnent a/s=1,2 et 1,9; b/g=0,05 et 0,06, se rangent ainsi à côté des *Kloeckera*, dont le taux d'alcool maximum ne dépasse pas 6°,8. *Zygosaccharomyces polymorphus* se place à côté des *Saccharomyces* glycologènes: a/s=2,2, b/g=0,09, et, comme ces *Saccharomyces*, donne des vins très alcoolisés (14°,3).

LES LEVURES DE VIN DE LA GIRONDE ET LEURS CARACTERES PHYSIOLOGIQUES

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Au cours des vendanges 1951, 1952, 1953, des prélèvements systématiques dans une trentaine de vignobles du Médoc, tant sur les grappes sur pied, que dans les cuves au moment du départ de la fermentation, ont permis d'étudier la flore des levures. Sur 432 souches isolées de 25 moûts en 1951 et 1952, voici la répartition des espèces trouvées :

Kloeckera apiculata 249, *Saccharomyces ellipsoideus* 138, *S. uvarum* 12, *S. Chevalieri* 6, *S. fructuum* 5, *S. Bayanus* 3, *S. Italicus* 1, *S. Steineri* 1, *Torulopsis bacillaris* 6, *T. famata* 5, *Rhodotorula mucilaginosa* 4, *Pichia fermentans* 2.

La fermentation vinaire est due à l'association des levures apiculées, toujours présentes, et des *Saccharomyces*. Parmi les *S. ellipsoideus*, quelques souches très alcoogènes : 32 (13°-14°), 5 (14°-15°), 4 (15°-16°) et 2 (16°-17°).

Les bilans des produits secondaires de fermentation ont été déterminés sur une trentaine de souches - a étant le nombre de molécules d'acide acétique, s d'acide succinique, h d'acétaldéhyde, b de butylèneglycol, g de glycérol, toutes ces levures vérifient, en anaérobiose, aux erreurs d'expérience près, l'équation :

$$5 s + 2 a + h + b = g$$

a/s varie de 1,5 à 3,4 b/g de 0,07 à 0,11 chez les *S. ellipsoideus*; chez les autres *Saccharomyces*, a/s varie de 2 à 3, b/g de 0,07 à 0,13.

Chez les *Kloeckera*, en anaérobiose, a/s varie de 1,2 à 2,4, b/g de 0,02 à 0,05. Toutes ces levures, malgré les différences morphologiques, fermentent le sucre de même façon. Seul *Torulopsis famata* fermente le sucre d'une façon particulière : a/s=0,1 à 0,2 b/g=0,03. Il faut noter qu'en aérobiose, les *Kloeckera* forment des doses relativement élevées d'acide acétique. Un cru déterminé possède souvent une race particulière de *S. ellipsoideus*, reconnaissable à la colonie géante.

Ces différences de races mises à part, la flore des levures est en France du même ordre que la flore trouvée par T. CASTELLI dans les vins italiens.

PHYSIOLOGICAL STUDIES WITH *VERTICILLIUM DAHLIAE*

by M. KAMAL and R. K. S. WOOD

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Preliminary studies have been made of the pectic enzymes and toxins secreted by an isolate of *Verticillium dahliae* obtained from cotton. The fungus was highly pathogenic, causing a typical wilt disease of young cotton plants when cultures were added to the soil in which they were growing.

Liquid cultures of the pathogen were investigated as follows. Protopectinase activity was estimated from determinations of the time taken for standard slices of cucumber fruit or turnip root to lose coherence when immersed in cell-free culture fluids. Toxicity to parenchyma cells was assessed by placing similar slices in cell-free filtrates and estimating the time taken 50 % of the cells to die, the criterion of death being taken as the inability of a cell to plasmolyse. The wilting activity of solutions was estimated by placing the cut ends of 3-4 week old cotton plants in the solutions and measuring the time taken for the leaves to show definite wilt symptoms.

Protopectinase was secreted in a variety of liquid media; somewhat greater activity was obtained in the presence of a low methoxyl pectin. Cell-free preparations had greatest macerating activity in the range pH 8.0 - 8.5. There was some loss of activity on dialysis which was only partially restored by adding salts. The enzyme was readily precipitated from solution with acetone or ethanol with little loss of activity at suitable concentrations. It was relatively heat-resistant, some 5 % of the original activity of solutions remaining after treatment at 100° C for 30 minutes.

When tested on slices of cucumber fruit or turnip root the toxicities of solutions were in general related to their protopectinase activity, e. g. a preparation which macerated slices of turnip root in 10-15 minutes, killed 50% of the cells in 20-25 minutes and autoclaved solutions neither caused maceration or killing within 240 minutes. Heating at lower temperatures however produced anomalous results. Thus, a preparation heated at 100° C for 15 minutes macerated slices of turnip root in 60 minutes but only relatively few cells were killed even after 240 minutes. In contrast, untreated solutions diluted so as to macerate slices in 60 minutes killed the majority of the cells in 100 minutes. Heat treatment therefore produced some separation of the macerating and killing substances.

Cell-free filtrates of cultures on suitable media readily caused wilting of young cotton plants when the cut ends were placed in the solutions for eight hours. The wilt which then occurred was irreversible, no recovery ta-

king place when the shoots were transferred to water. The plants recovered however if the ends of the stem were removed; the length necessary depended on the period the stem had been immersed in the active solution.

Wilting was greatly delayed if the plants were kept in the dark or under conditions when the loss of water by transpiration was reduced; such plants rapidly wilted on transfer to conditions favouring transpiration. The loss of water from plants with their cut ends in water or in active solutions was similar; the uptake of water from active solutions was however considerably reduced.

Active solutions lost little activity after autoclaving or dialysis so that solutions having little or no protopectinase activity may cause wilting. On the other hand, the disease symptoms in the presence of protopectinase more closely resembled those observed in naturally infected plants.

There is therefore some evidence that in culture filtrates, the main agents of wilt are high molecular weight compounds which produce their effect by impeding water uptake; in this respect the effect of protopectinase may be secondary. Under natural conditions the action of protopectinase on plant tissue might produce substances having a similar physiological action.

THE PRODUCTION AND PROPERTIES OF PECTIC ENZYMES SECRETED BY *FUSARIUM MONILIFORME*

by R. K. SINGH & R. K. S. WOOD

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The fungus *Fusarium moniliforme* is an economically important parasite of a wide range of plants. It attacks many members of the Gramineae and also occurs on such different hosts as potato tubers, apple and tomato fruit and cotton plants. Because of its wide host range and the ease with which it is cultured, this fungus was considered a promising subject for physiological investigation and, in particular, for studies of pectic enzyme secretion since it typically causes a soft rot of plant tissue. The work to be reported is mainly concerned with an analysis of the nutritional factors affecting production of pectic enzymes and with the properties of these enzymes.

The activity of the various pectic enzymes was estimated as follows.

Protopectinase : rate of loss of coherence of standard slices of potato tuber.

Pectinesterase : rate of formation of carboxyl groups with a high methoxyl pectin as substrate.

Polygalacturonase : rate of formation of reducing groups or rate of loss of viscosity in solutions of pectin or sodium pectate.

Active protopectinase preparations were obtained when the fungus was grown on a variety of natural media e. g. extracts of tomato fruit, turnip root or potato tuber, but when apple juice or a number of synthetic media were used there was little or no activity although growth was good in most cases. There was no correlation between enzyme secretion and mycelial growth; active solutions were sometimes obtained from cultures in which growth had been poor.

The addition of relatively small quantities of a tomato fruit extract to synthetic media gave very active protopectinase preparations; similar results were obtained when pectin was added. This effect was examined more fully with a glucose, asparagine and mineral salts solution. Protopectin, pectin, sodium pectate, sodium ammonium pectate and galacturonic acid all gave active solutions; galactose, gum arabic, gum tragacanth, alginic and mucic acid were ineffective. On the basis of these results a simple synthetic medium consisting of pectin, glucose, asparagine and mineral salts was evolved which after supporting growth of the fungus for seven days at 25° C gave very active protopectinase preparations causing loss of coherence of slices of potato tuber 0.5 mm. thick in 10-15 minutes. It is interesting to note that the addition of apple juice to this medium greatly reduced the activity of the culture solution at the same time producing little effect on growth.

The protopectinase secreted under these conditions is rapidly inactivated in the temperature range 50-60° C, has an optimum pH in the range 8.0-9.0, is dialysable with little or no loss of activity, is inhibited by phosphate at 0.02 M and above and is readily precipitated from aqueous solution by the addition of acetone or ethanol.

These protopectinase preparations had very low pectinesterase activity and reduced the viscosity of solutions of sodium pectate in an anomalous manner, producing a relatively slow and steady fall rather than the very rapid reduction generally observed. The viscosity reducing enzyme was also more active on solutions of pectin than on those of sodium pectate; its other properties closely resembled those described above for protopectinase.

When assayed by reducing group determinations, the protopectinase preparations were more active on solutions of sodium pectate than on pectin, particularly in the early stages.

Preliminary chromatographic examination of the breakdown products obtained by the action of these protopectinase preparations on solutions of pectin or sodium pectate (derived from the pectin by enzyme de-esterification) indicate that a different series of compounds are produced suggesting that more than one enzyme systems may be involved in the degradation of different pectic substances. On the basis of the above evidence it would seem that the pectic enzymes secreted by *Fusarium moniliforme* are different from those described from other organisms.

III. Mycologie médicale

THE EFFECT OF 1,2,3,4-TETRAHYDRO-FLUORENONE ON FUNGAL PATHOGENS

by Katherine S. WILSON

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and

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The antifungal properties of 1, 2, 3, 4-tetrahydro-fluorenone (THF) were first noted by us in 1950 during a screening program in which new compounds synthesized by N. V. Organon, Oss, Holland, were tested against species of *Candida*, *Cryptococcus*, *Microsporium* and *Trichophyton*. Subsequent investigations of the effect of THF on the growth of a variety of fungi were carried out with both plant and human fungal pathogens.

In vitro studies with *Trichophyton mentagrophytes*, *T. rubrum*, *Microsporium audouini*, *Cryptococcus neoformans* and *Candida albicans* showed that these organisms were highly sensitive to THF. However, the outstanding feature of our results was that the action of THF on these fungi was not only strongly inhibitory but also fungicidal. This has been indicated by repeated unsuccessful attempts to subculture 7 and 10 days after treatment. The test organisms varied somewhat in their sensitivity to THF. *T. mentagrophytes*, *T. rubrum* and *M. audouini* were markedly inhibited by this compound, the activity against *C. neoformans* was slightly less, and *C. albicans* was the least sensitive. THF is only sparingly soluble in water. However, it was found that with 5 per cent Tween-80 as a solvent, inhibition was increased by approximately 100 per cent. Because of the limited quantity of THF available, an extensive search for an effective solvent was not possible, and standard potency curves could not be constructed at this time. However, an indication of relative activity was obtained by comparisons with sodium lauryl sulfate, sodium benzene dodecyl sulfonate, propionic, caprylic and undecylenic acids, and a number of other anionic surface-active compounds with known inhibitory properties.

Although THF was considerably less soluble, it was more effective than the other compounds tested. This was illustrated by one of our first tests in which weighed quantities of the crystalline compound (2 mg.) were placed on the agar surface. After 7 days most of the THF remained undissolved but the degree of inhibition was still significantly greater than that of the most active compound, although the latter was completely dissolved.

THF was less promising as a possible fungicidal agent for phytopathogens. Five species of plant fungi, *Gibberella zeae*, *Botrytis cinerea*, *Cladosporium fulvum* and two unidentified species of *Fusarium*, were tested. With these, inhibition was only temporary followed by growth after 6 days in the presence of concentrations of THF which permanently inhibited our test species of human pathogenic fungi. However, toxicity studies carried out with six crop plants, including turnip, cabbage, tomato, rape, wheat, and radish suggested a high degree of tolerance to THF at all stages of growth. It is therefore possible that derivatives might be found of greater toxicity to the fungi without injuring the host.

These tests, though only preliminary, have shown that THF merits further exploration as a promising antifungal agent, particularly for certain fungi which are pathogenic to man.

[We understand that our preliminary findings have, in essence, been confirmed and extended through further *in vitro* studies, and that clinical tests with THF are in progress under the direction of N. V. Organon (personal communication, Dr. J. Lens).]

UNTERSUCHUNGEN ZUM PATHOGENITÄTSNACHWEIS VON CANDIDA ALBICANS

von D. JANKE

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Das zweite Hauptthema befasst sich mit dem Uebergang saprophytärer Keime in pathogene Erreger. Vom Standpunkt des Dermatologen kann zu dieser Frage ein Beitrag zunächst nur insofern geleistet werden, als er über klinische Beobachtungen berichtet, von denen aufgrund kultureller Untersuchungen anzunehmen ist, dass für sie sonst saprophytäre Keime krankheitsverursachende Bedeutung haben. Die ursächliche Bedeutung des aus den Hauterscheinungen gezüchteten Keimes kann mit Sicherheit nur bewiesen werden, wenn die KOCH'schen Postulate erfüllt sind. Diese Beweisführung ist aber im allgemeinen bei den hier in Frage stehenden Erregern (z. B. hefeähnliche Pilze der Gruppe *Candida*) nicht möglich, weil das Angehen

der Infektion offenbar weitgehend an Besonderheiten des Makroorganismus gebunden ist.

Mein eigener Beitrag besteht nun darin, bei Patienten mit krankhaften Erscheinungen, aus denen hefeähnliche Pilze gezüchtet wurden, einen spezifischen Antikörper im Serum nachzuweisen. Hierdurch wird wahrscheinlich gemacht, dass im speziellen Fall der im allgemeinen saprophytäre Keim eine pathogene Bedeutung hat. Der Beweis hierfür ergibt sich aus der Tatsache, dass die Serumveränderungen als reaktive Erscheinungen des Organismus auf einen solchen Keim gedeutet werden müssen, mit dem der Körper sich auseinandergesetzt hat.

Résumé. — Recherches sur la preuve de la pathogénie de Candida albicans.

Grâce à la mise en évidence d'anticorps spécifiques dans le sérum des malades offrant des symptômes d'affections d'où l'on a isolé des champignons levuroides, on peut établir, dans un cas particulier, qu'un germe vivant généralement en saprophyte, présente vraisemblablement une valeur pathogénique.

La preuve résulte de la propriété pour le sérum modifié de se comporter en réactif de l'organisme : il doit permettre d'interpréter la valeur du germe avec lequel l'anticorps a réagi en déterminant la précipitation.

DIMORPHISME PARASITAIRE ET DIMORPHISME SAPROPHYTIQUE DE L'*HISTOPLASMA DUBOISII* VANBREUSEGHE 1952

par R. VANBREUSEGHEM

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L'*Histoplasma duboisii* a été découvert en 1952 chez un malade européen qui avait séjourné quelques années au Congo Belge et qui présentait des lésions ganglionnaires. L'auteur de cette note l'a étudié et décrit comme une nouvelle entité spécifique. Il a reconnu à ce champignon pathogène deux aspects morphologiques différents à l'état parasitaire : l'un, qu'il nomme forme *capsulatum*, est absolument semblable à celui qui caractérise un champignon pathogène très voisin, *Histoplasma capsulatum* ; l'autre, la forme *duboisii*, est particulière à ce champignon pathogène et absolument distincte de la forme *capsulatum*. L'étude de la bibliographie a révélé que 8 cas semblables avaient été décrits antérieurement et un cas nouveau d'origine nigérienne a été publié après l'observation princeps de l'auteur. En

culture, la souche isolée par l'auteur n'a pu jusqu'ici être obtenue que dans la phase mycélienne. Mais les souches de Duncan et de Walker peuvent, sur les milieux spéciaux usuels, reproduire à la température de 37° la phase levure. Expérimentalement, le champignon à l'état saprophytique dans sa phase mycélienne s'est révélé doué d'un pouvoir pathogène beaucoup plus élevé que le champignon à l'état parasitaire dans sa forme *duboisii*. Le cobaye, par la voie intratesticulaire, le hamster, par la voie intrapéritonéale, réagissent par des lésions rapidement évolutives. La souris a pu être inoculée avec succès par la voie nasale avec la phase mycélienne.

La signification exacte de la forme *capsulatum* n'a pu être précisée jusqu'ici. Elle n'apparaît qu'après l'inoculation de la culture dans sa phase mycélienne et disparaît rapidement pour être remplacée par la forme *duboisii*.

L'auteur propose que les expressions « état parasitaire » — parasitic state — et « état saprophytique » — saprophytic state — soient utilisées pour distinguer chez un même champignon le parasite du saprophyte; que les mots « phase mycélienne » — mycelial phase — et « phase levure » — yeast phase — désignent les divers aspects morphologiques d'un même champignon à l'état saprophytique et que le mot « forme » accompagné d'une épithète ou d'une expression appropriée, désigne un certain aspect morphologique particulier de l'état parasitaire d'un champignon. Ainsi peut-on reconnaître au *Sporotrichum schenckii* un état parasitaire et un état saprophytique; une phase mycélienne en culture à 25° et une phase levure en culture à 37° sur le milieu de Francis; enfin, dans les tissus, à l'état parasitaire, une forme en navette, une forme cryptococcus et une forme astéroïde.

TEIGNE DU MOUTON

par GUILHON (J.) et CHAUVIER (G.)

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La teigne du mouton est une mycose cutanée, contagieuse, provoquée par le développement de plusieurs dermatophytes, plus spécialement dans les parties du corps non couvertes de laine.

L'affection signalée et décrite pour la première fois par CHABERT, en France, sous le nom de dartre, en 1783, a été retrouvée depuis par divers auteurs français et étrangers.

Elle se manifeste par des néoformations croûteuses qui s'agrandissent, progressivement, pouvant atteindre jusqu'à 2 à 3 centimètres de diamètre, qui régressent lentement.

CATANEI a montré que le mouton pouvait être parasité par deux espèces de dermatophytes : *Trichophyton pruinosum* n. sp. et *Trichophyton discoides* SABOURAUD, 1909. Plus récemment, en 1950, L. ECHENIQUE, a observé un *Microsporum* non déterminé. Enfin, en 1953, nous avons isolé d'une lésion de teigne ovine *Ctenomyces granulosis* (SABOURAUD, 1908).

La teigne du mouton, d'origine saprophytique ou parasitaire a été reproduite expérimentalement.

Le diagnostic clinique est le plus souvent facile. Toutefois, il faut éviter des confusions avec la gale sarcoptique, l'ecthyma contagieux et des lésions de la toison provoquées par divers germes mal classés se rapprochant des actinomycètes.

En résumé, la teigne du mouton est une affection contagieuse, qui a été peu étudiée et qui paraît pouvoir être provoquée, d'une façon certaine, par au moins trois espèces de dermatophytes : *Trichophyton pruinosum* CATANEI 1931, *Trichophyton discoides* SABOURAUD, 1909 et *Ctenomyces granulosis* (SABOURAUD, 1908).

SPOROTRICHOSE DU CHIEN

par GUILHON (J.) et OBRY (J.)

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La sporotrichose du chien est une mycose gommeuse, rare, provoquée par la multiplication d'un hyphomycète sporotriché : *Rhinocladium Schenckii* HEKTOEN et PERKINS, 1900. Décrite pour la première fois, en France, par GOUGEROT et CARAVEN, en 1907, elle a été constatée depuis cette époque par MEYER (1915), COLLET (1934), GUILHON et OBRY (1953).

Cette mycose s'observe le plus souvent chez des sujets jeunes, de sexe mâle. Elle se manifeste d'ordinaire par des gommès, plus rarement par des cordes lymphatiques.

Les gommès localisées ou disséminées se développent dans la peau, plus rarement dans les viscères (poumon, foie), les muqueuses et les séreuses. Elles peuvent se ramollir, puis s'ulcérer et se fistuliser et donner issue à une sérosité peu visqueuse, roussâtre. Certaines d'entre elles s'indurent et restent fermées. La sporotrichose évolue sans fièvre, ni réaction ganglionnaire, s'il n'y a point de complications bactériennes.

Sa terminaison peut être fatale ou heureuse avec ou sans traitement.

Au début de son évolution elle doit être distinguée de certaines formes de staphylococcies cutanées et d'histiocytomes ulcérés.

Le champignon responsable de l'affection apparaît sous la forme de conidies-levures à l'intérieur des macrophages ou à l'état libre dans le pus des gommès.

La sporotrichose cède à une thérapeutique iodée précoce.

INFLUENCE OF CERTAIN ENVIRONMENTAL FACTORS ON THE GROWTH AND SPORULATION OF THE DERMATOPHYTES

by Heiti PALDROK

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The growth rate of the dermatophytes is highest at a temperature of about 30°C. The temperature most favourable for conidia development seems, however, to lie some degrees below that of the optimum for growth. The range for conidia development is wider below than above the optimum for sporulation. The number of the conidia mounts slowly in accordance with temperature rise above zero, but after having passed the maximum it sinks rapidly. Also the shape of the conidia, and especially that of the macronidia, on which latter the botanical classifications have been based, follows, on the whole, the numeral occurrence of the structures. At favourable temperature ranges the shape of the macroconidia is classical or normal. But it is less differentiated or becomes retrogressive in unfavourable conditions. Closer to the temperature limits for growth the retrogression process in cultures may go even further, the cultures becoming deprived of aerial mycelium at all. The colonies then turn glabrous, and the only structures developed are chlamydospores. The retrogressive forms of macroconidia have often been interpreted as independent form-types and have thus served as base for creating of new, but actually non-valid species or genera. The form changes of the macroconidia as well as transformations of the colony growth type can be effectuated also by other physico-chemical factors, inter alia, by toxic compounds, such as fungicides or fungistatics.

THE MIGRATING NUCLEUS

by E. SILVER KEEPING (née DOWDING)

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It is well known that some species of *Mucor* possess (+) and (—) strains. Each is sterile when grown alone, but when a (+) and a (—) strain are paired in a petri dish, fruit-bodies appear along the line of meeting of the two mycelia. What must happen is that when (+) and (—) hyphae

fuse, the (+) and (—) nuclei come together and fuse in pairs to initiate the formation of fruit-bodies.

In *Gelasinospora tetrasperma*, after a (+) mycelium is paired with a (—) mycelium the resulting fruits are not always limited to the line of meeting of the two strains but may also be evenly distributed all over one or other of the two strains, for example all over the (+) mycelium. Such distribution suggests that nuclei have passed out of the (—) mycelium into the (+) mycelium, converting it from the homokaryotic (—) state to the heterokaryotic or ($\pm$) state. Experiments have confirmed this supposition and shown that nuclei may migrate through a mycelium at the rate of about 8 mms. per hour.

Photographs both of living, and of fixed and stained mycelia, show a morphological difference between stationary and migrating nuclei.

ASPECT MORPHOLOGIQUE DE QUELQUES CHAMPIGNONS PATHOGENES AU MICROSCOPE ELECTRONIQUE

par R. C. ZAPATER (*) et M. Malfatti

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L'étude des champignons suivants a été faite à l'aide du microscope électronique : *Candida albicans*, *C. tropicalis*, *C. krusei*, *C. parakrusei*, *Histoplasma capsulatum*, *Paracoccidioides brasiliensis*, sous leur forme levure et leur forme mycélienne. D'autre part, *Nocardia asteroides*, *N. brasiliensis* et *N. madurae* ont également été étudiés. Les observations au microscope électronique (modèle E.M.U. agrandissement 4000x) ont permis de voir dans les formes levures et mycéliennes des condensations cytoplasmiques dont l'interprétation nécessite de nouvelles recherches cytochimiques.

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SECTION 20

PHYTOPATHOLOGIE

1. — TYPES DE POURRITURES DES BOIS TROPICAUX SPECIALEMENT PROVOQUEES PAR LES POLYPORES

TYPES OF ROTS IN TROPICAL TIMBERS

by W. P. K. FINDLAY

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RAPPORT

The subject of decay of forest trees and converted timber in the tropics has so far received very little attention. Only a few outstanding diseases, such as that of *Juniperus procera* caused by *Fomes demidoffii*, have been recognised and described.

A number of workers in Europe, including Roger HEIM and MARTINEZ, have contributed valuable papers on lignicolous fungi of the tropics. In South America, TORREND, TEIXERA and JORGE WRIGHT have described some of the *Polyporaceae* of that region.

The fungi that attack trees and timber in India have been studied by BAGCHEE at Dehra Dun, and numerous papers on the *Polyporaceae* of Bengal have been published by S. R. BOSE of Calcutta, while HASAN has recently begun a study of these fungi in Pakistan.

However, no comprehensive work dealing with forest and timber fungi of any tropical region has yet appeared.

In Europe many kinds of wood are now received from the tropical regions and it is inevitable that decay should occasionally be found in the imported logs and lumber. Fungi can often be isolated from such timbers but unless we happen already to have available in our collections cultures of the same species, we often cannot identify them. It seems very desirable that surveys of the prevalence and severity of heart rot in the principal timber trees should be undertaken in the countries of origin.

A serious obstacle to progress in these studies is the absence of floras dealing with the higher Basidiomycete fungi of tropical regions. Another difficulty is the confused state of taxonomy of *Polyporaceae* at the present time. For instance, CUNNINGHAM's excellent work on New Zealand Polypores uses a nomenclature that is unfamiliar to most mycologists and

different from that employed elsewhere. It would be most useful to all workers in this field if floras of *Polyporaceae* and later of wood-inhabiting *Agaricaceae* and *Thelephoraceae* could be prepared for the principal forest regions in the tropics. The fungal flora of the world does not vary to the same extent as the Phanerogamic one and probably one flora would be adequate for a region as large as the whole of West Africa. I would like to appeal to systematists to collaborate in the preparation of such floras, adopting some agreed taxonomic system which, for the present, might have to be a rather conservative one.

We have received at Princes Risborough specimens of fungi collected by Forest Officers in various territories and, with the assistance of the authorities at Kew and elsewhere, have succeeded in identifying a number of them, but so far none of these Forest Officers has developed a keen interest in the fungi comparable to that in insects, which has led to a number of Forest Officers becoming competent entomologists. I think it is the absence of books of reference combined with the difficulty of preserving specimens of fungi which so discourage the beginner in this field.

The plant pathologists in tropical countries are generally so occupied in dealing with day-to-day problems concerning the prevention of disease in agricultural crops that they have had little time to consider tree diseases, I find that there is among plant pathologists a pessimistic attitude towards tree diseases which they feel cannot readily be controlled. It is true, of course, that one cannot economically spray forest trees or exercise many of the direct methods of control that are available in dealing with diseases of annual crops.

Experience in Europe has, however, shown that good silvicultural treatment may delay the onset of heart rot and that the correct choice of species for any particular area may largely avoid the risk of subsequent disease. While it is unfortunately true that few indigenous tropical forests can as yet be described as subject to any silvicultural management at all; in many areas, notably in East and South Africa, large areas of exotic species of *Cupressus* and *Eucalyptus* have been planted and already present pathological problems.

From a practical viewpoint the most important information about the incidence of heart rot is the age at which it first becomes significant and at what age loss due to decay exceeds the annual growth increment. Data of this kind are available for some of the major tree species in British Columbia and Oregon U.S.A. BOYCE, for example, has shown that in certain stands of Douglas fir the rot increment begins to exceed the gross increment some time between 300 and 350 years. I do not know of any similar studies on important tropical timber trees. It is likely that the onset of serious decay may occur at a comparatively early age in many of the less durable tropical species. Recently I saw samples of *Eucalyptus saligna*

from coppice-grown shoots only 14 years old that contained both heart rot and so-called « brittleheart ». In some species the deterioration due to « brittleheart » may be more significant than that due to fungal attack, as it may occur in much younger trees.

In any intensive study of the heart rot in a species it is necessary to make cultures from the infected tree trunks. One must not assume that decayed wood having the same appearance as that associated with any particular fungus has necessarily been caused by that fungus. In British Columbia, for example, heart rot in *Thuja plicata* had always been attributed to *Poria weirii* until BUCKLAND made numerous isolations of the causal fungus, when he discovered that the decay of the cedar in the interior of the Province is in fact caused mainly by *Poria albipellucida*, which causes a rot which is very similar to that brought about by *Poria weirii* which is common in the coastal region. Before such exhaustive studies of this type can be made it is necessary to have available cultures of all the principal heart fungi of the region. Thanks to the assistance given by U.N.E.S.C.O. and to the activities of the permanent committee of the British Commonwealth Collections of Micro-organisms, catalogues of the principal collections of fungi have been published, so that investigators can now find out what species are available in culture. Most of the collections still contain a comparatively small proportion of the wood-rotting fungi of the tropics and I would appeal to anyone who isolates any of these fungi in culture to deposit sub-cultures in one of the national culture collections.

A much wider range of species is likely to occur on felled and converted timber than as parasites in standing trees and we cannot hope in our laboratories in Europe to maintain in culture and be familiar with *all* the species that are likely to occur, but since there are many cosmopolitan species and many saprophytes are widely distributed in both the New and Old World, we can at least get to know some of the principal species.

For example :

Polystictus sanguineus appears to be one of the commonest of all wood-rotting fungi in the tropics.

Lenzites palisoti (= *L. repanda*) and *Polystictus occidentalis* are two other species that seem to occur very commonly.

Schizophyllum commune is probably the commonest fungus on logs imported into this country but it causes little damage to the wood. It has been observed that it is sometimes able to survive temperatures used in kiln-drying wood.

Lenzites trabea and *L. striata* are two brown rot fungi that seem able to endure long periods of storage in air-dry wood and which have therefore been isolated on a number of occasions from imported timber.

Though it is outside the scope of this discussion, I would like to refer

to the importance of preventing sap-stain (blue stain) in logs of light coloured woods shipped to Europe from the tropics. Most of this stain appears to be due to species of *Diplodia*, mainly *Lasiodiplodia theobromae* and *D. natalensis*.

Practical investigations of the rate of development of wood-rotting fungi under tropical conditions and of means for preventing the deterioration of timber shipped from the tropics in log form are needed.

It is comparatively easy by proper handling to prevent fungal deterioration of sawn timber but to ensure that logs of susceptible species of wood arrive in a sound condition in Europe and U.S.A. is a far more difficult problem.

VARIATIONS AMONG ISOLATES
OF *FOMES ANNOSUS* (FR.) CKE.
OF DIFFERENT GEOGRAPHICAL AND HOST ORIGIN

by Harold S. McNABB, Jr.

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The fungus, *Fomes annosus*, exhibits very diversified geographical and host ranges. Different degrees of pathogenicity are also present within these ranges. All these factors have led to the belief that strains exist within this species. Attempts in such isolate grouping have been previously made. This study, of which a part is presented in this paper, dealt with a new attempt using different criteria on a larger number of isolates of wider geographical and host distribution.

From the isolates collected, 42 were arbitrarily selected for intensive studies. The origins of these isolates represented 23 host species and the geographical regions of Western North America (18), Eastern North America (15) and Europe (9).

Of the criteria used, pH adaptation in synthetic, liquid medium and wood decay under pure culture conditions produced interesting results in light of past observations as to the nature of this organism under natural conditions.

The pH experiments were not devised to present optimum pH growth values, but instead to indicate the ability of this organism to change the pH of its substratum during growth. The initial pH of this substratum, a liquid medium, was 4.6. Final pH after six weeks of fungous growth ranged from 3.4 to 6.2 for this group of isolates. A majority of them were capable of raising the initial pH to a value greater than 5.2.

The decay abilities of the isolates were determined by pure culture decay tests on the sapwood and heartwood of *Pinus resinosa*. Twelve of the isolates caused a dry weight loss of more than five percent in one or both of these types of test blocks during the given exposure time.

No definite correlation could be made between the origin of the isolates and any of the results of the previous experiments. The study shows the individuality present within the species, *Fomes annosus*.

2. — LES COMPETITIONS FONGIQUES DANS LES AFFECTIONS DES PLANTES CULTIVÉES

CAUSES OF VARIATION IN WHEAT YIELD

by Mary D. GLYNNE

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Grain yields of winter wheat ranged from about 4 to 49 cwt per acre (*5 to 61 m. quintals per hectare) in field experiments at Rothamsted during the fifteen years between 1938 and 1952. My paper attempts to measure the major factors responsible for this great variation. Weather and cultural conditions, of course, are important; not only because they affect yield directly, but also because they influence other things which affect yield, of which some of the most important are foot and root rotting fungi, lodging and weeds.

The effects of varying weather (and some other factors) in different years, is measured by the range of annual yields of plots where wheat is grown each year with the same treatment. On Broadbalk field, Squarehead's Master wheat is grown with various manurial treatments, each plot receiving the same fertilisers each year; the plots are divided into 5 sections, of which one, in rotation, is left fallow for one year. Annual comparisons are made of the 8 representative plots surveyed, which include the least and the most fertile plots in the experiment. The difference between the highest and the lowest yield of individual plots ranged from 7.5 to 25.4 cwt. per acre; for most plots the ratio of highest to lowest yield was between 2 and 3 (only occasionally higher), showing that yields in the best years were between two and three times as high as in the worst years. Similar ratios between highest and lowest occurred on wheat grown each year on the 4 and 6 course rotation experiments on two other fields.

The lowest yields were produced from crops grown without nitrogenous fertiliser on land where wheat (or barley) had been grown in successive years on the same land. On Broadbalk, yields of these plots fell to 4 and 5 cwt. per acre in the worst years; but some produced 15 and 16 cwt. per acre in the best years. The poor yields on land without fertilisers were in some years further decreased by root damage caused by take-all, *Ophiobolus graminis*; and every year tillers were killed and straws weakened by eyespot, *Cercospora herpotrichoides*. The incidence of both diseases varies widely year by year and their effects on yield are obvious.

* (1 cwt. per acre = 1.255 m. quintals per hectare).

During the 15 year period, yields of the Broadbalk unmanured plot, on the 3 sections following wheat averaged 9.4 cwt. per acre. Another plot receiving complete fertiliser with nitrogen yielded twice as much; one with very heavy dressings of dung averaged 23 cwt. per acre. Lodging then set a limit to further increase in yield. Nitrogenous fertilisers almost abolished losses from take-all but increased the weight of straw and the proportion of plants severely infected by eyespot at harvest, both of which increased lodging.

Fallowing almost doubled the yield of the unmanured plot, but the effect lasted for only one year. Fallow also produced smaller increases on the more fertile plots. Fallowing acted primarily by increasing soil fertility, but it also reduced weeds and take-all, all three factors having their greatest effect on the least fertile plots. Fallow reduced the proportion of straws weakened by eyespot; but it also increased the weight of straw; the first effect decreased, the second increased the extent of lodging; so that in some years and in some plots there was more lodging in the sections after crop, in others after fallow. Again lodging limited yield which, on the dunged plot reached a maximum average of 28.6 cwt. per acre.

The effect on wheat of one, two and three preceding years under crops other than wheat or barley, on land already infested by the eyespot and take-all fungi were tested in two experiments completed in 1951 and 1953, both seasons in which eyespot was below average on the classical experiments. One year under other crops had a similar but rather greater effect than one year's fallow had on Broadbalk; yields were increased, eyespot reduced and take-all almost eliminated. A break of two years further increased yield and decreased eyespot; a third year produced relatively little additional benefit. Yields after two and three year breaks under various crops averaged 36 cwt. with a maximum of 40 cwt. per acre.

Eyespot, but not take-all, can be reduced by spraying wheat in early spring with sulphuric acid; this also reduces weeds. Spraying has little effect on height or weight of straw but greatly reduces eyespot, and, therefore, lodging. It is most effective on the most fertile plots, because, with less lodging they can give higher responses to fertilisers. Spraying has increased the yields by different amounts in different years, and its effect depends partly on fertilisers and seed rate, but increases have been as much as 10 cwt. per acre.

The choice of variety, of course, has a great effect on yield; Square-head's Master and Bersee, with the same favourable treatments, yielded respectively 34 and 49 cwt. per acre.

COMBINED INFECTION WITH ROT-CAUSING BACTERIA AND FUNGI

by K. A. SABET

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Several combinations of rot-causing bacteria and fungi included *Rhizopus nigricans*, *Pythium (ultimum)*, *Botrytis allii*, *Phytophthora infestans*, *Alternaria tenuis*, *Aspergillus flavus*, *Bacillus polymyxa*, *B. pumilus*, *B. subtilis*, *B. megatherium*, *Bacterium carotovorum*, *B. atrosepticum*, *B. aroideae*, and *Pseudomonas marginalis*.

A special technique was adopted in which potato slices and rarely onion bulb slices, were used as a medium for inoculation. The slices were kept under aseptic conditions in contact with water in sterile Petri dishes and generally incubated at 30°C. Extent of rot was determined as the difference between the *actual* weight of the slices before inoculation and the *actual* weight of unaffected tissues after a given period.

Synergism was found to exist between certain bacteria and fungi such as *B. polymyxa* plus *R. nigricans* and *Ps. marginalis* plus any of the components used, whether fungi or bacteria.

Antagonism or competition, was found between *P. (ultimum)* plus *B. polymyxa* or *B. pumilus*.

Most of the other components reacted indifferently and the amount of rot produced by their combinations equalled more or less, that produced by the more active organism.

Under less favourable conditions, certain components react as synergists e.g. *B. carotovorum* plus *B. polymyxa* incubated at 35°C; and *R. nigricans* plus *B. polymyxa* at 96 or 100 % relative humidity. In these instances, synergism under the general experimental procedure was not apparent or less marked.

UN HYPERPARASITE DU *POLYSTIGMA RUBRUM* (PERS.) DC

par Mladen YOSSIFOVITCH

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Vers la fin de la végétation du prunier (octobre-novembre) en région centrale de Serbie (Choumadia) sur les stromas produits par *Polystigma rubrum* se développe une couche de nature mycelienne, de couleur rosée, qui les recouvre plus ou moins complètement.

Ce phénomène étudié depuis 1951, a permis de faire les constatations suivantes.

1. L'analyse microscopique de la masse rose de la surface des stromas révèle l'existence des organes de reproduction d'un *Trichothecium* pouvant appartenir à l'espèce *T. roseum*. Les mesures des conidies nous ont donné les dimensions suivantes: extrêmes: $12,5 - 19,2 \times 6,6 - 9,9 \mu$; moyennes $15,3 \times 8,0 \mu$. Ces dimensions sont très proches de celles fournies par Saccardo et ne diffèrent pas beaucoup de celles que VIENNOT-BOURGIN (1949) a établies pour les conidies du même champignon développé sur des coussinets de *Nectria cinnabarina*.

Avec les conidies prélevées sur des stromas de *P. rubrum*, nous avons réussi à contaminer des pommes tavelées.

2. Avec des conidies obtenues en culture pure, il nous a été très facile de contaminer des stromas normaux de *P. rubrum*, en pulvérisant des feuilles portant ces stromas à l'aide d'une suspension de spores. En nature, il nous a été également possible, au mois de novembre, d'augmenter le nombre des stromas atteints par *Trichothecium* en les humectant avec de l'eau contenant une suspension de spores.

3. La présence du *T. roseum* a pour conséquence la stérilisation des stromas atteints, la nécrose des éléments ascogènes du mycélium du *Polystigma rubrum*, contenus dans ces stromas.

4. Un filtrat liquide d'une culture de *T. roseum* est sans effet sur le développement des Stromas et des conceptacles de *Polystigma rubrum*.

Conclusion. — 1. *Trichothecium roseum* se comporte comme un véritable hyperparasite. Les stromas parasités restent stériles. L'action inhibitrice ici rapportée est identique à celle manifestée lors de son développement sur les périthèces de *Gnomonia veneta*.

2. Cette action inhibitrice n'est pas due à la substance antibiotique que *T. roseum* est capable de produire.

3. Etant donné le nombre important des stromas affectés par *T. roseum*, il nous paraît possible d'entrevoir une lutte efficace contre *Polystigma rubrum* en provoquant la dissémination des conidies de l'hyperparasite à l'automne dans les vergers de pruniers.

THE INFLUENCE OF SOIL ENVIRONMENT ON THE MORTALITY OF MAIZE GRAINS

by J. L. HARPER
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The cultivation of maize in Northern temperate countries is dependent on early planting to make the most of the limited growing season. Early plantings however, usually give poor emergence and varieties differ in

their ability to germinate and produce healthy seedlings after sowing in cold wet soils.

Experiments have been made to determine which factors of the soil environment are responsible for this mortality, to assess the influence of mechanical damage on the liability of grains to pre-emergence mortality, and to determine the nature of the resistance shown by different maize varieties and hybrids.

Two contrasted maize varieties, Wisconsin 275, a cold hardy North American hybrid, and Virginian Withe Horsetooth, a Southern late ripening open pollinated maize, were planted in the field at weekly intervals throughout the spring. Emergence from these plantings was inversely proportional to the time between sowing and emergence, and this time was related to the number of hours below 10°C , which the grains experienced after sowing. Soil was sampled from the field at weekly intervals and maize grains planted in it were exposed to a temperature of 5°C for 10 days and subsequently to a temperature of 20°C to allow rapid germination. It was found that the mortality caused by this cold experience varied between sampling dates. These variations were positively correlated with soil moisture content, as also was the proportion of this mortality which occurred after the start of germination. Cold moist soils appeared to predispose the surviving grains to post-germination mortality. However, even when the cold experience was maintained constant and allowance was made for the correlation of mortality with soil moisture content, there still remained a significant variation in mortality from one sampling date to another. This probably reflected variations in the activity and abundance of soil pathogens not directly related to the temperature and moisture content of the soil.

At soil temperatures below 0°C there is true frost damage, unaffected by seed dressing with fungicides. Above 0°C however, there is mortality of a form controllable by fungicides, which increases to a peak between 5 and 10°C and falls off and becomes negligible between 15 and 20°C . Previous germination for 1-6 days at 20°C significantly increases susceptibility to true cold but not to pathogenic damage.

Experiments were made in which soil moisture content and temperature were both controlled over a range 0 - 30°C and from soil sticky point to a moisture content too low to allow germination. After exposure of maize grains to these conditions for 9 days, ungerminated grains were transferred to damp sand at 20°C to test for continued ability to germinate. Grain mortality was greatest at 8°C in the wettest soil and at 30°C in the soils too dry to allow germination.

Under those conditions which just did not allow germination, mortality was very high and falling moisture or temperature below this level led to increased survival.

These laboratory observations agree closely with observations in the field, both for spring plantings in Northern temperate regions, when the grains meet cold wet soil, and for the mortality which has frequently been observed to follow sowing in hot dry soil in monsoon countries, when the monsoon rains have been delayed.

In all our tests, at both high and low temperatures, White Horsetooth has proved far more susceptible to damage than Wisconsin 275. However, the difference between the varieties was reduced by piercing the pericarp above the embryo before exposure of the grains to cold wet soil, and varietal differences were completely eliminated if the pericarp was removed. It has also been found that these and other varieties differ in the time taken for emergence in the field. The earlier the planting the greater the difference between varieties in this respect. As the mortality is largely dependent on the time spent in the soil before emergence, slower germination will lead to higher mortality. When the time taken to emerge is considered in determining the influence of planting date on mortality, the differences between varieties becomes insignificant.

Breeding for new varieties of maize for expansion of growth of this crop into colder regions of shorter growing seasons should involve search both for innate resistance to pathogenic attack, which is largely located in the pericarp, and for ability to germinate at low temperatures.

In recent years, American and European workers have adopted cold tests for selection of hardy strains of maize. These involve exposure of the grains in soil to low temperatures for 5 days or more, and subsequent transference to a higher temperature to allow germination. The emergence from these tests gives a measure of the resistance of maize varieties to seedling blight. However, the « cold test schedule » varies greatly from worker to worker, both in respect of temperature, of exposure time, and of the degree to which soil moisture content is controlled. For these tests to be of more than local value, rigid standardization must be adopted, and it is our experience that water supply during the test is the most important factor to stabilize. However, when the conditions of temperature and moisture of soil samples are standardised for these tests, variations in the pathogenic potential will still be responsible for differences in test results from one sampling date to another. It is very important that cold tests of this nature should always be conducted with reference to one standard hard shelled maize variety, so that account can be taken of the uncontrollable variations in rigidity of the test.

In view of the results obtained from exposure of grains to high temperatures in dry soil, it is advisable to develop a test incorporating this type of hazard in the testing of maize varieties for hot countries with sporadic rainfalls.

3. — PROBLEMES DE L'INCUBATION DANS LES MYCOSES

L'INCUBATION D'UNE MYCOSE

par H. KERN

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RAPPORT

L'incubation d'une mycose comprend la période qui s'écoule entre l'infection et l'apparition, sur la plante, des premiers symptômes extérieurs. Durant ce laps de temps, l'agent infectieux se développe à l'intérieur de l'hôte; sous son influence, la plante hôte subit de grandes transformations physiologiques: son métabolisme se modifie, ses réactions de défense entrent en jeu, son plasma s'altère.

Ces altérations s'accroissent de plus en plus; les réactions cellulaires deviennent si distinctes que leurs conséquences, les symptômes extérieurs, en sont visibles. En pathologie végétale, il est plus difficile de déterminer le moment précis où l'incubation prend fin et où la maladie éclate: des sensations telles que la douleur, la fièvre, etc. ne nous servent pas d'indicateur. La délimitation de l'incubation restera donc toujours un peu vague. Nous nous demandons cependant: que se passe-t-il dans la plante pendant cette phase de préparation de la maladie?

Le jeu si complexe des interractions entre l'hôte et son parasite est difficile à étudier et malgré son importance peu connu; pour le moment, nous ne pouvons qu'esquisser quelques problèmes.

1. — *La durée de l'incubation.*

L'extension du parasite dans son hôte dépend largement de leur biochimie et de leur structure réciproques. De ces deux facteurs dépendent les voies que suivra l'infection, sa localisation finale, et la longueur de la période d'incubation.

Bien des parasites ne quitteront jamais la cellule ou le tissu contaminés; les produits de leur métabolisme atteindront les cellules adjacentes, et les effets seront rapides et intenses, mais limités. D'autres agents répandront leurs toxines dans la plante; ces toxines peuvent soit frayer un passage à l'agent infectieux, soit provoquer directement l'intoxication de la plante. Les parasites d'un troisième groupe pourront se maintenir dans leur hôte sans l'induire à des réactions bien vives; leur métabolisme est toléré par l'hôte.

2. — *Le métabolisme du parasite dans l'hôte.*

Un parasite des vaisseaux pourra réduire, plus ou moins, le passage

de l'eau par la seule présence de ses hyphes (trachéomycoses). Mais en général, ce sont les échanges de substances entre parasites et hôtes qui amènent les réactions cellulaires pathologiques. Le parasite peut former ou libérer des *enzymes* (p. ex. pectinases), des *toxines* (p. ex. toxines de flétrissement), des *substances de croissance* (p. ex. l'acide indolacétique, nécrohormones) etc.

Les résultats obtenus avec des produits du métabolisme de certains parasites *in vitro* permettront bientôt des recherches approfondies sur les réactions dans la plante infectée.

3. — *Le stade initial de la maladie de l'hôte.*

La maladie de l'hôte débute dans son protoplasme; les symptômes extérieurs (souvent peu spécifiques) ne sont que la conséquence de ces réactions cellulaires préliminaires. La connaissance de ces processus ne fait que débiter. On a étudié, par exemple :

a) les altérations des systèmes enzymatiques.

La patuline s'unit aux groupes -SH du plasma. L'acide fusarique inhibe les échanges respiratoires des levures et des tissus de tomate.

b) les perturbations du bilan de l'eau.

Certaines toxines fongiques détériorent les couches périphériques du plasma et modifient leurs propriétés osmotiques. La plante flétrit par suite d'une intoxication de son plasma.

c) les réactions de défense.

Une partie des réactions de l'hôte se dirige spécifiquement contre le parasite. Le plasma est en principe capable de se défendre (faiblement en général) mais il est souvent difficile de le prouver. La cellule hôte peut aussi modifier ses capacités de croissance (méristèmes induits, démarcations) ou même répondre par la production d'anticorps.

Ces processus initiaux provoquent d'abord des symptômes physiologiques généraux (élévation de la température, troubles du métabolisme, etc.), puis les symptômes morphologiques typiques de la maladie.

4. — *Les modifications du parasite dans son hôte.*

Les médecins discutent actuellement les modifications que subissent les bactéries pathogènes de l'homme (formes filtrantes) et par suite les modifications de leur résistance aux phages etc. Les champignons pathogènes des plantes subissent aussi de profondes transformations pendant leur vie parasitaire, soit dans la morphologie de leurs cellules et noyaux (formation de suçoirs etc.), soit dans leurs capacités physiologiques.

Ainsi se forme une communauté entre l'hôte et son parasite dès que l'infection a pris pied. L'étude expérimentale de leurs interréactions primaires presque invisibles nous permettra de comprendre un peu mieux les stades de l'évolution d'une maladie végétale.

COMMUNICATIONS DIVERSES

THE SUSCEPTIBILITY
OF WHEAT AND BARLEY VARIETIES
TO LOOSE SMUT (*USTILAGO NUDA*) IN ENGLAND

by C. C. V. BATTS

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A large number of wheat and barley varieties are being tested at Cambridge for susceptibility to the disease. The first inoculations were made in 1950 using the hypodermic needle method (POEHLMAN, 1945). Results were satisfactory but the method is rather slow and in succeeding years the partial vacuum method (MOORE 1936, OORT 1939) has been used. About eight ears of each variety to be tested are inoculated with each source of smut, and from these, a number of ear rows are sown in the field.

With wheat varieties, consistent results have been obtained and the method indicates their relative susceptibilities. The maximum infection obtained is about 70 % of the plants in very susceptible varieties such as Vilmorin 27, Hybrid 46 and Fylgia. Slightly susceptible varieties such as Atle, Alba and Victor give about 6 % infection. In between is a range of infections on other varieties. For each variety the numbers of infected and healthy tillers on each plant are counted and there is a strong tendency for the plants of very susceptible varieties to contain a higher percentage of infected tillers than the less susceptible varieties. For example in one season Vilmorin 27 had over 90 % of the tillers infected whereas Alba had about 25 % infected tillers. This agrees with the findings of OORT (1947). A number of varieties such as Nord Desprez, Cappelle Desprez, Jubilegem and Scandia appear to be highly resistant.

At least two physiologic races of the fungus are present in wheat varieties. Bersee, Eclipse, Pilot and Yeoman appear to be susceptible to race 3 only. The very susceptible varieties mentioned above are susceptible to races 2 and 3. Seed of differential varieties for race identification was obtained from Prof. OORT and the determination of races was made by the use of his keys (OORT 1947). A collection of the smut from Vilmorin 27 made in 1952 appeared to consist of a mixture of races.

The testing of barley varieties is presenting difficulties not encountered in the testing of wheat varieties. Most of the seed from the barley varieties inoculated in 1951 failed to germinate. This phenomenon has been reported by several other workers. The 1952 inoculations were more satisfactory and the results for eight varieties are summarised in Table 1.

Table 1

Results of eight spring barley varieties inoculated with two sources of smut.

<i>Variety</i>	<i>Smut from spring barley</i>	<i>No. of ear rows infected</i>	<i>Smut from winter barley</i>	<i>No. of ear rows infected</i>
A	26 %	2/4	46 %	3/5
B	36	3/4	13	2/4
C	28	3/5	12	1/4
D	36	5/5	19	2/4
E	24	2/2	14	2/4
F	16	1/4	9	4/4
G	0	0/5	0	0/4

Note : The percentages refer to number of plants infected.

The table shows that with one exception the highest infection is obtained after inoculation with smut from spring barley, indicating the possible existence of more than one race. A disturbing feature is the variation in the number of rows infected. As far as could be judged all ears were inoculated at about the same stage, i.e. early anthesis. It is a well known fact, however, that pollination in certain varieties takes place while the ear is still enclosed in the sheath. Special attention was given to the stage of development in the 1953 inoculations. It appears that the time of inoculation is more critical in barley than in wheat; in testing wheat varieties it is unusual for an ear of a susceptible variety to escape infection.

In Canada the examination of embryos, particularly of barley, has been proceeding for some time so that information on the amount of smut in a seed stock may be obtained. Embryo examination is also being done at Cambridge, most attention being given to wheat. There appears to be no sharp line of demarcation between resistant and susceptible varieties as far as presence of mycelium in the grain is concerned. Evidence is accumulating to indicate that in certain resistant varieties the fungus does not penetrate the developing ovary, in others it penetrates and may get even into the scutellum as in susceptible varieties. Further work is being done along these lines.

There is much to be learned about the biology and development of the loose smut fungus in wheat and barley before the testing of varieties can be satisfactorily undertaken.

CROWN RUST AND STEM RUST ON OATS IN ISRAEL.

by I. WAHL

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Severe damage caused annually to oat varieties cultivated in Israel by crown rust (*Puccinia coronata avenae* (Cda) Eriks. and Henn.) and stem rust (*P. graminis avenae* (Pers.) Eriks. and Henn.) made it imperative to explore the possibilities of introducing resistant varieties and to determine the identity of physiologic races of both rusts, their prevalence and distribution. Hence, oat observation nurseries were established in various regions of the country. Some of them have comprised a number of varieties utilized as parental material in oat breeding for crown rust and stem rust resistance in different countries.

It was ascertained that epiphytotics of crown rust begin in March, reach their climax in April and decline in May. The outbreak of stem rust occurs by mid-April and becomes very extensive in May.

Santa Fe and its derivatives were completely vulnerable to crown rust. The same was true of Bond hybrids in most nurseries. Some varieties descended from Victoria, like Boone, Cedar, Tama and Vicland performed considerably better than Branch, Missouri 0 — 205, Neosho and Osage. Branch and Missouri 0 — 205 rusted badly in all nurseries. A number of Minnesota selections of the Landhafer x (Mindoo x Hajira-Joanette) crosses were relatively promising.

Identification studies of the physiologic races of crown rust have revealed that two unusually virulent races, never previously reported, predominated in 1952-53. One of these produced infection types 3 or 4 almost on all hosts of the old and new differential sets, so that only Glabrota and Saia were immune from its attack. The other race was innocuous on Bond, Glabrota and Saia and virulent to the remaining differentials. The Crown Rust Research Laboratory of the U.S. Dept. of Agriculture, located at Ames, Iowa, has designated the first race as race 276 and the second as race 270.

Stem rust reaction field trials have shown that only varieties possessing the so — called Canadian factors of resistance were slightly infected, while White Russian and Richland-Rainbow derivatives rusted heavily. Saia thus far proved to be the only variety immune from both rusts in the fields. Races 6 and 8 of stem rust were the most prevalent and widespread. The local race 6 produced in the greenhouse pustules of types 1 and 2 on canuck, Clinton x Ukraine (C. I. 6537), Garry Sel. (R. L. 1697.27) and saia seedlings.

The ubiquitous wild grass — *Avena sterilis* L. and early sown cultivated varieties under irrigation became infected by the described crown rust and stem rust races already in November. These early epiphytotics seem to assist in the multiplication and dissemination of oat rusts in Israel.

INFECTION EXPERIMENTS WITH *CORYNEBACTERIUM RATHAYI* (E.F.S.) DOWSON

by K. A. SABET

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A séries of inoculation experiments were carried out to determine the mode of infection with *Corynebacterium rathayi* which is suspected as the cause of the yellow slime disease of cocksfoot grass (*Dactylis glomerata*). The organism was inoculated into the leaves and inbetween leafsheaths of *D. glomerata* and *Triticum vulgare* either alone or in association with *Anguina tritici* the cause of the eelworm disease of wheat. The organism, or the combination of the bacterium and the eelworm were also inoculated into the soil in which both hosts were then grown. Inoculations were further made with the slime produced on affected wheat plants either alone or in association with eelworms.

Infection took place in young seedlings of wheat grown in soils infested with both *C. rathayi* and *A. tritici*. Symptoms produced were similar to those of the cocksfoot grass disease.

Inoculations made into *D. glomerata* with either the bacteria, the eelworms, the slime or any of their combinations were all unsuccessful.

It is suggested that an obligate association may be existing between *C. rathayi* and a particular eelworm capable of infesting *D. glomerata* parallel to the association found between *C. tritici* the cause of the yellow slime disease of wheat and the eelworm *A. tritici*.

RELATIONS BETWEEN PROTEIN METABOLISM OF POTATO LEAVES AND THEIR SUSCEPTIBILITY TO LATE BLIGHT (*PHYTOPHTHORA INFESTANS*)

by Gerhard GRUEMMER

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It is known since a long time that late blight, caused by *Phytophthora infestans*, does not appear in all seasons of the year. The attack does normally not start before July or early August. The reason for the appearance of late blight not before mid-summer has been interpreted differently; a meteorological and a physiological theory have have been outlined in the past.

The *meteorological interpretation* assumes that certain environmental conditions are necessary for a successful infection of potato leaves by the fungus, conditions not always realized. The closing of potato-rows in mid-summer is supposed to cause a suitable humidity which is thought to be the most important climatic factor for the outbreak of *Phytophthora*. Several authors tried to find out the conditions necessary for successful infections. A survey of literature would show, that results have been widely different with regard to temperature as well as to suitable humidity for the parasite conidia.

The *physiological theory* on the other hand claims that susceptibility of host tissue for late blight is largely dependent on the tissue's metabolism. Young leaves are supposed to be resistant (although not entirely immune), whereas the leaves of old and ripening plants turn more and more susceptible.

The date of first attack on leaves is corresponding with important changes in leaf physiology, which — according to my investigations — are due to the beginning of subterraneous tuberformation, which is a heavy strain for the leaves of the whole plant. At this time proteins are decomposed and derived from leaves to tubers. This important physiological change is known as ageing or « age-chlorosis » of leaves. It starts in the eldest leaves and later on concerns the higher inserted leaves as well. These chlorotic leaves are physiologically quite different from normal young leaves with prevailing protein synthesis.

Leaves of young growing potatoes or tomatoes can easily be transferred into chlorosis by a blackout. Within a few days the leaves have turned chlorotic; this can be proved by determination of protein and chlorophyll content.

To prove how far the metabolism of leaves can affect the results of *Phytophthora*-infections, young tomatoes were turned chlorotic in this way and were inoculated with *Phytophthora*. Controls were normal green plants of the same age. After inoculation both were exposed to the same temperature and humidity. The physiological condition of the tissue was exerting an important influence upon the results of infection; chlorotic leaves were heavily infected, whereas the normal green leaves suffered only slightly from disease.

In summer 1953 sprouts were taken out of potato fields in regular intervals and their protein and chlorophyll contents were analysed. This was done to establish, at what time the decomposition of proteins and chlorophyll was starting and how far it was correlated with the first attacks of *Phytophthora* in the same fields.

Extensive investigations showed that the attack of *Phytophthora* started, when the decomposition of proteins in the leaves had begun. The first lesions were noticed on the lowest leaves; at this time their decomposition

of proteins had come into action, but had not yet affected the higher inserted leaves of the stem. Several weeks later the protein dissimulation reached the higher inserted leaves, and consequently these were shortly afterwards attacked by the fungus as well. Non-chlorotic normal green leaves were never attacked in any stage of disease development.

The influence of leaf physiology on the degree of *Phytophthora* damage could also be noticed by observations on normal and virus-infected plants. Potatoes infected by leaf-roll virus were damaged three times heavier than normal plants under the same conditions.

My communication is not to establish the extreme opinion that the physiological character of leaves is exclusively responsible for the outbreak of the disease. During observations in last year it was noticed that not all leaves affected by beginning chlorosis were attacked by the parasite at once. Sometimes one or two weeks passed away, before a new wave of *Phytophthora*-infections damaged all leaves that had turned susceptible in the meantime. Without any doubt that delay was due to climatic factors limiting the development of the parasite.

The result of my observations and investigations, which could only briefly be reviewed, is a compromise between the extreme « meteorological » and the purely « physiological » theory of *Phytophthora* outbreak. Two factors are necessary: Firstly the intrinsic factor (susceptibility of leaves owing to beginning chlorosis) and secondly the extrinsic factor (suitable temperature and humidity for a mass production and germination of conidia resp. zoospores).

Susceptibility of leaves is necessary for a successful attack of late blight; how far these susceptible tissues actually are infected, is largely dependent on climatic factors. The cooperation of the intrinsic and the extrinsic factors is determining the outbreak and progress of the disease.

PHOMA FOVEATA EN TANT QU'AGENT D'UNE POURRITURE DES TUBERCULES DE LA POMME DE TERRE

par H. BRAUN

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Au printemps 1953, a été observée, pour la première fois en Allemagne, la pourriture des pommes de terre causée par *Phoma foveata*. La maladie se caractérise par des lésions concaves à contour plus ou moins circulaire, isolées ou confluentes, pouvant être confondues avec des taches d'*Alternaria*. Ces taches s'accroissent et se fendillent en leur centre.

Au dessous des taches la chair est colorée en brun-noir sur quelques mm. de profondeur.

En 1940, FOISTER (Edinburgh) a décrit le champignon (*Phoma foveata* sp. nov.). L'examen d'une souche de cette espèce avec l'espèce trouvée en Allemagne montre l'identité des 2 parasites. Les essais jusqu'ici réalisés déterminent un optimum de température relativement bas pour la maladie, soit aux environs de 10°C, tandis que l'optimum de température pour la croissance du mycélium dépasse 20°C. Cette différence importante est interprétée comme étant en relation non pas avec le parasite mais avec l'hôte ou plus exactement avec les réactions déterminatives de l'infection.

Les variétés de pomme de terre se montrent différemment sensibles sans qu'il y ait de relation avec l'état de développement du tubercule.

Il est remarquable de constater que cette maladie altère uniquement les tubercules en magasin, visible seulement vers le mois de janvier. Elle constitue un « vice caché » (« verdeckter Mangel ») qui en automne, au moment de la livraison, n'est pas perceptible. Les pommes de terre malades par *Phoma foveata* sont en général impropres à servir de tubercule-semence.

PHYTOPHTHORA CACTORUM EN TANT QU'AGENT DE LA POURRITURE DU COLLET DU POMMIER

par H. BRAUN

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Durant l'été 1950 a été observée pour la première fois en Allemagne une pourriture de l'écorce du pommier, et principalement sur Reinette Cox Orange type IX. Elle se caractérise comme suit : les arbres âgés de 10 ans croissent et même fleurissent normalement, puis, brusquement se dessèchent après avoir parfois manifesté tout d'abord une coloration plus claire du feuillage. On observe sur ces arbres, très près du bourrelet de greffe, une écorce colorée en bleu-violet, les tissus sous-jacents étant brun-foncé suivant une zone bien délimitée.

En 1951 a été isolé un champignon. Des essais d'infection ont pu reproduire les symptômes typiques qui s'identifient à ceux de *Phytophthora cactorum*. Depuis, la maladie s'est rapidement répandue en Allemagne et menace sérieusement l'arboriculture fruitière. Elle était auparavant connue aux Etats-Unis, au Canada, en Argentine et Nouvelle-Zélande. récem-

ment elle est signalée en Angleterre, Hollande et Belgique où elle cause de graves dégâts.

Le parasite s'installe par des blessures du tronc causées par la gelée ou des accidents mécaniques. Il vit dans le sol et se trouve transporté par le vent et la pluie. La plupart des porte-greffes semblent résistants. Indépendamment de la variété Cox, plusieurs variétés cultivées sont sensibles.

Tout récemment des fraisiers ont été gravement atteints par le champignon de sorte que cette culture sous les arbres fruitiers augmente les dangers de contamination.

La lutte est rendue d'autant plus difficile du fait que la maladie est décelée souvent trop tard et qu'alors l'excision profonde du tissu infecté jusqu'au tissu sain ne peut plus mener à des résultats satisfaisants.

THE SPREAD OF *ARMILLARIA MELLEA* IN ORCHARDS AND FRUIT PLANTATIONS

by R. W. MARSH

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A major difficulty in plotting the spread of an attack by *Armillaria mellea* is that the fungus grows almost entirely in the underground portions of its hosts. Attacked plants often show no symptoms above-ground until shortly before their death. When woody plants are infected the progress of the disease is usually slow, so the pattern of spread of the disease can only be determined by observations continued for many years.

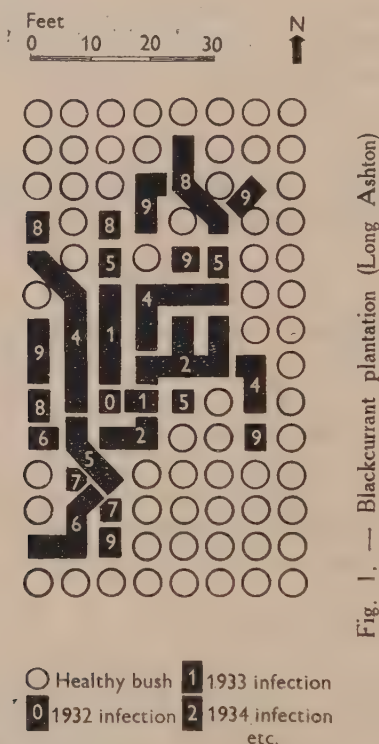
A. mellea on blackcurrant bushes.

Records were kept for ten years in a plot of Baldwin blackcurrants at Long Ashton, planted at 6 × 6 ft. Fig. 1 shows the positions of the bushes killed by *A. mellea* in each successive year. Infection did not spread concentrically but extended along lines of contiguous bushes. The spread north- and-south (which was the direction in which the plot was cultivated) was more rapid than the spread east- and-west.

The results suggest that the important factor in the spread of the fungus was contact of the roots of a healthy bush with the roots of an adjacent diseased bush. There was no evidence that the growth of rhizomorphs through soil unoccupied by roots played any important part in extending the infection.

A. mellea on apple trees.

The records on apples were not taken on dessert or culinary varieties on vegetatively-reproduced rootstocks but were made on cider varieties grown in grass orchards as standard trees on seedling stocks. The observations were made in the West of England, where, as in Normandy and Brittany, each cider orchard contains trees of many varieties and many ages. In these orchards, when a tree dies from old age or disease, a young tree is often planted in approximately the same position.



Armillaria mellea is common in these orchards but its spread is more difficult to trace than in blackcurrants, as the roots are not accessible, so the fungus cannot be located until it appears in the bark at the base of the trunk.

Orchard near Glastonbury (see Fig. 2). In this orchard of 160 trees, records were taken for 13 years, ending in 1951. In the plan (Fig. 2) the trees are classified according to age: the large circles represent the trees planted before 1908, the medium-sized circles show the 1908-23 plantings

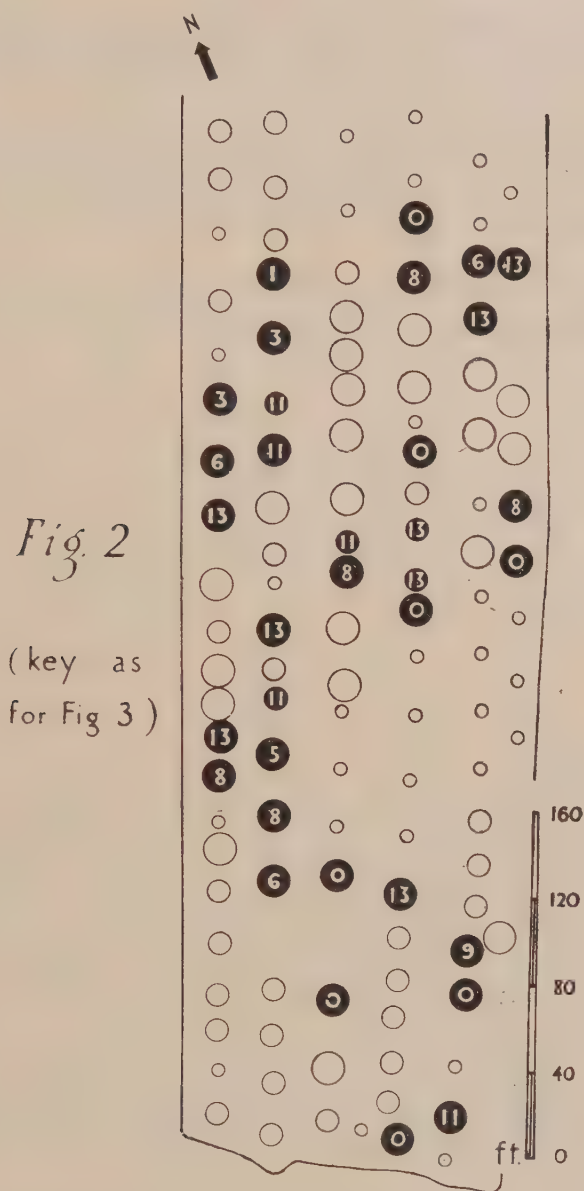


Fig. 2. — Cider orchard (Glastonbury)

and the small circles the trees planted 1923-38. Trees killed by *A. mellea* in each of the successive years from 1938 to 1951 are indicated by the figures 0 to 13 respectively.

The records show that the progress of the disease was slow and the pattern of spread erratic. The deaths showed no relationship to the variety of tree, but there was a connexion with age of tree and with proximity to earlier infections. The details are summarized in Table 1.

TABLE 1. — *Glastonbury orchard*: records of *A. mellea* infections in relation to age of tree and proximity to older infections.

Tree planting date	Records 1940 - 51					
	Records 1938 - 39		Trees < 70 ft. from 1938-9 infn.		Trees > 70 ft. from 1938-9 infn.	
	No. of trees	In- fections recorded	No. of trees	In- fections recorded	No. of trees	In- fections recorded
Before 1908	48	10	35	19	3	1
1908-23	67	2	44	12	21	0
1923-38	45	0	37	0	8	1

These observations show that, between 1940 and 1951, more than half the trees over 30 years of age were killed by *A. mellea*; approximately 20 % of the trees between 15 and 30 years old were killed; but only 1 of the 45 trees under 15 years of age. The table does not include twenty-two trees planted as refills between 1938 and 1951. Although many of these trees were planted in the positions from which *Armillaria*-killed trees had been removed, not one has so far been recorded as attacked by *A. mellea*.

Orchard near Taunton (see Fig. 3). Fig. 3 represents a cider orchard near Taunton, the trees being shown in the same age groups as in Fig. 2. Records in this orchard were also maintained for 13 years (1938 to 1951) and the deaths from *A. mellea* attack in the successive years are represented as before.

In the Taunton orchard there were no trees more than 70 ft. from a 1938-39 infection but the effect of proximity is evident from the figure. The effect of age of tree is summarized in Table 2.

Dates of planting :

- before 1908
- 1908 - 1923
- since 1923

Trees killed by *Armillaria mellea* :

- in 1938
- in 1939, etc.

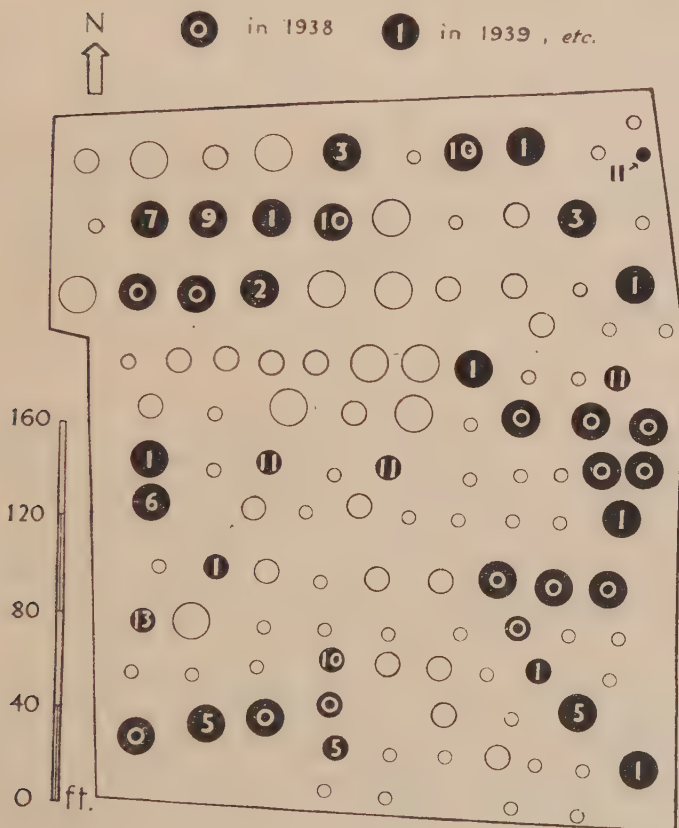


Fig. 3

TABLE 2. — *Taunton orchard: records of A. mellea infections in relation to age of tree.*

Tree planting date	Records 1938 - 39		Records 1940 - 51	
	No. of trees	Infections recorded	No. of trees	Infections recorded
Before 1908	37	20	17	9
1908-23	33	5	28	7
1923-38	49	0	49	1

The records from the Taunton orchard corroborate those from the Glastonbury orchard in showing the susceptibility of the older trees and the resistance of those under 15 years of age. Between 1938 and 1951, thirteen additional trees were planted as refills: none of these have shown attack by *A. mellea*.

Discussion.

In making the observations on apple trees it was often noted that infections was found on part of the base of the trunk 2 or 3 years before the encirclement of the trunk by the fungus brought about the death of the tree. This slow progress of the attack suggests that the invasion of the root system is also gradual and might proceed over a number of years. It is possible that in a young vigorous apple tree the fungus in the roots makes little progress but with increasing age, the resistance of the tree to attack declines.

The pattern of spread in the apple orchards surveyed cannot be fully explained, but the data on proximity suggest that, as in blackcurrants, the extension of the roots into an *Armillaria*-infected site plays an important part in initiating infection. However, in standard apple trees on seedling roots, it is evident that the period from the beginning of infection to the death of the tree may cover several years. In an orchard where an infected area is adjacent to trees differing greatly in age and vigour the pattern of spread of *Armillaria* infection will be irregular, and its future direction cannot be predicted with certainty.

BREEDING RED CLOVER FOR RESISTANCE TO CLOVER ROT DISEASE

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INTRODUCTION

This disease is caused by the Ascomycete *Sclerotinia trifoliorum*. Control measures are difficult to apply and breeding of resistant strains appears the best solution. In Europe, several have been bred, e.g. the Swedish Merkur and Resistentia. These are semilate, whilst in Britain a double cut Broad Red type is required for 4-course rotational areas where one year leys are sown. Existing early strains are more susceptible than the lates. Three approaches are open to the breeder:

1. To select from within established local varieties. Selections would be adapted already to local conditions.
2. Selection on a wider basis, and hybridisation with local varieties.
3. Interspecific crosses with other *Trifolium* species.

The first two only are considered here, since too little is known about interspecific relationships in this genus.

MATERIAL AND METHODS

A greenhouse technique has been found more reliable than field selection. The inoculum was prepared by transferring young cultures to flasks of moist sterile bran which was later dried, powdered and stored at 1-2°C. The plants, either in pots or seedling boxes were stood on moist peat and after dusting with inoculum were covered with polythene frames. For each experiment both plants and inoculum were of the same age, and the same amount of inoculum was used throughout. Since there is no evidence of biologic races, the most virulent isolate collected was used. Plants were removed for observation and marking when all but a few had been killed. For large scale selections young seedlings, 50 per earthenware pot standing in water were inoculated at the third trifoliate leaf stage and covered with a small bell jar. Survivors were potted and when mature split into four to provide three replicates for inoculation and one for crossing.

RESULTS

1. *Selection from indigenous strains.*

22 seedlings survived out of 33,000 Essex Broad Red inoculated. These were tested when mature with 22 unselected controls of similar age and history. There were three groups of significance. The most susceptible Group I contained 16 controls and 8 selected plants, Group II 6 controls and 11 selected and Group III 3 selected plants only. The effect of selection is evident though several seedling 'escapes' had undoubtedly been included. The 10 best and one resistant control were diallel crossed giving 55 families. These, plus families from the susceptible controls and resistant X susceptible plants were sown 30 per box thinned to 25 before inoculation 4 months later. Owing to shortage of seed there were 2 replicates only per family. The percentage survival was calculated, and from this the mean percentage for each parent. The mean for the 55 families was 14.7 %, compared with 1.3 % for the 3 control families, with a range from 0 % to 84 %. The mean for families from control X selected plants was 5.7 %. 6 parents were especially good, with many families having a survival rate of 50 %. Families from the remainder were much poorer, but were little worse than those from crosses of these with good parents.

Previous experience has shown that 6 basic plants are adequate for strain building (WATKIN WILLIAMS & W. Ellis DAVIES — unpublished data.). 3 plants from each of 6 families derived from a chain cross between the 6 good parent plants were selected and intra and inter family crosses made. The best progeny will be polycrossed and the third generation compared in the field with the original Essex Broad Red from which the basic plants were selected.

2. *Selection from other populations of red clover.*

The centre of origin of the genus *Trifolium* is supposedly the N. Mediterranean. Here, one would expect the most genetic variation, especially amongst wild unselected populations. Samples of 12 of these were obtained embracing an area from S. Spain to Turkey and inoculated together with Essex Broad Red and English wild red clovers as controls. All were better than the indigenous wild red, but 3 only, from Sicily, Granada, and Versailles, were more resistant than the cultivated control. Susceptibility increased progressively northwards and eastwards.

The best plants from these 3 populations were crossed with Essex Broad Red clover in an attempt to combine resistance with the other characters required.

DISCUSSION

No immune types have been found, and in view of the non-specific nature of the pathogen it is possible that they do not exist. Resistance is not inherited in a Mendelian fashion, but the data suggest that the overall resistance of a strain can be improved by careful selection and breeding. In view of the apparent prepotency of susceptibility when crosses between susceptible and resistant plants are made the greatest care is needed to eliminate any susceptible plants from the breeding stock. The possibility of finding more resistant material amongst wild populations from the gene centre has not yet been explored fully, but appears to offer a fruitful line of enquiry.

THE HOSTRANGE OF *GLOMERELLA CINGULATA*
WITH SPECIAL REFERENCE
TO *COLLETOTRICHUM LINDEMUTHIANUM*

by J. A. von ARX

Baarn, Netherlands

Many fungi have been described under the names of *Collectotrichum*, *Gloeosporium* and *Glomerella*, which differ very slightly in morphological aspects. They cause anthracnoses, leafspots and fruitrot on cultivated plants. One-spore-cultures and inoculations have been made with isolations from different plants. The cultures showed a wide variation of growth characters. In Petri-dishes sector formation was often observed. Several isolations from the same hostplant were found to be morphologically different, for instance in the color of the sporemasses and in the size of the spores and these characteristics altered during the cultivation, especially after a passage through the living hostplant.

Cross-inoculations were often successful. For instance a strain with large spores, isolated from a leafspot of the orchid *Oncidium excavatum*, caused an anthracnose on young plants of *Phaseolus vulgaris*. After 5 passages this strain proved to be very virulent for bean and affected the variety « Kieviet Bulten », which was resistant against 6 different strains of *C. lindemuthianum*.

From these and other such experiments it must be concluded that a number of fungi, described as species of *Collectotrichum* and *Gloeosporium* are only forms of a changeable, polyphagous and cosmopolitic species. This species should be named *Glomerella cingulata*. The conidial stage belonging to *Collectotrichum* consists of about 700 synonyms.

ESSAIS DE LUTTE CONTRE LE «SCHWARZWERDENS», NOIRCISSEMENT DES JEUNES POUSSES DE MUGUET (*SCLEROTIUM DENIGRANS* PAPE)

par H. PAPE

Biologische Bundesanstalt f. Land- und Forstwirtschaft Kiel-Kitzeberg

1°) Il est tout d'abord donné une courte description des manifestations externes et du développement de la maladie des jeunes pousses du Muguet de mai (*Convallaria majalis* L.) désignée entre autres termes : « Schwarzwerdens » : noircissement, « Schwarzer Tod » : mort noire, « Schwarzer Pest » : peste noire.

2°) Sont ensuite étudiés les facteurs qui favorisent le développement actif de l'agent pathogène : *Sclerotium denigrans* Pape.

3°) Seront abordés les moyens et les mesures de lutte et de protection contre la maladie. Dans cette partie l'auteur exposera principalement les recherches qu'il a entreprises concernant la lutte contre le champignon par traitement des jeunes pousses et du sol au moyen des procédés chimiques (composés mercuriques, composés dérivant du chloronitrobenzène et autres fongicides). Il communiquera ensuite les différents résultats obtenus.

A CONTROL PROGRAM FOR THE WILT DISEASE OF *MENTHA PIPERITA* L. CAUSED BY *VERTICILLIUM ALBO-ATRUM*, R. ET B.

by C. L. PORTER

Department of Biological Sciences

Purdue University, West Lafayette, Indiana, U.S.A.

The devastation caused by *Verticillium albo-atrum*, R. et B. in the mint fields of Michigan and Indiana has been a source of grave concern to those industries using the essential oils produced by *Mentha piperita* L.

Since 1946 The Beech-Nut Packing Co., one of the world's largest producers of Chewing gum has financed a research project in my laboratory with the object of establishing a satisfactory control program for the so-called « mint wilt disease ». Since that date a team of investigators has worked constantly studying the physiology, morphology, and etiology of the causal fungus; as well as the disease syndrome. This paper summarizes the results of our investigations to date.

We have isolated a virulent strain of *Verticillium albo-atrum*, R. et B. which we have designated as strain « B11B ». We have proved that this fungus strain can cause all the symptoms characteristic of the wilt disease of the peppermint plant.

In our studies we have established the following facts which will be elaborated in my paper to the Congress :

1. The host range of strain, B11B.
2. Strain, B11B can be made to attack the cortical layers of the roots of plants other than mint. We think this is the initial phase of an infection which eventually becomes vascular.
3. Strains of *V. albo-atrum* pathogenic to such crop plants as tomato, potato, egg plant, hops, cotton, etc. are not pathogenic to *M. piperita*.
4. The various methods by which the host become infected.
5. The rate of movement of the inoculum within the plant.
6. The thermal death point of the fungus as compared to the thermal death point of the host.
7. The fungus may be present in the host which may exhibit no symptoms. Therefore, the only certain method to ascertain whether a plant is infected is to isolate the fungus from the plant.
8. It has been impossible to isolate *V. albo-atrum* from soils in which crop debris is absent.
9. Isolations of *V. albo-atrum* can be made indefinitely from mint charges - previously inoculated with the fungus.
10. Following the inoculation of strain B11B into raw muck soil it was recovered subsequently for a period of only about two weeks.
11. Following the inoculation of strain B11B into sterile muck soil it could be recovered indefinitely.
12. We know the method of sclerotial formation in *Verticillium* and have germinated the sclerotia of strain, B11B.
13. The most marked symptoms of *Verticillium* wilt occur between 25° and 30°C.
14. The optimum temperature for the growth of *V. albo-atrum* on Czapek's solution again was determined as between 20° and 25°C.
15. The pH limits for the growth of *V. albo-atrum* were determined.
16. The capacity of the fungus to utilize C was studied. Eleven carbon sources were screened.
17. Both organic and inorganic sources of N. were studied in their effects in the nutrition of strain, B11B.
18. Under certain circumstances nitrite-N. can be utilized by strain, B11B.
19. Thirteen amino acids were tested singly and in combination as sources of N. for strain, B11B.

20. The effect of the trace elements, Cu, Zn, Al, Co, Cd and Ni on the growth and development of strain, B11B was investigated. Under greenhouse conditions, both cadmium and aluminium reduced the morbid effects of *V. albo-atrum* on peppermint.
21. Muck soils have been screened for organisms inhibitory to the growth of *Verticillium*. Several organisms have been isolated that give excellent promise of biological control.
22. The effect of chemical fumigants in repressing *Verticillium* attack is being studied.
23. An intensive histological and physiological investigation on the effect of *Verticillium* and its toxins on the host has been made resulting in a new theory as to the nature of pathological wilting.
As a result of data secured to date we conclude :
 1. That under certain environmental conditions the presence of *V. albo-atrum* in *Mentha piperita* will not seriously reduce the capacity of the plant to produce the essential oil.
 2. That *V. albo-atrum* can be reduced in the soil by cultural and biological control measures to such an extent that little or no injury will result to field-planted peppermint.

CONTRIBUTION A LA CONNAISSANCE DE L'EFFET DE BAKANAE DU RIZ

par Ch. STOLL

Institut de botanique spéciale de l'Ecole Polytechnique Fédérale. Zürich

Gibberella fujikuroi (Saw.) Woll. a été cultivé *in vitro* sur des milieux liquides, en variant les conditions de température et de nutrition.

Le contrôle de l'activité des filtrats provenant des cultures a été effectué au moyen des tests suivants :

1° Mesure de l'inhibition de la germination de chlamydospores d'*Ustilago zeae* (Beckm.) Ung.

2° effet du flétrissement sur des jeunes tomates par la méthode de GÄUMANN et collaborateurs.

3° Action bactériostatique par le test de diffusion sur des plaques d'agar.

Nous avons obtenu, après purification chimique, des cristaux blancs, ayant un point de fusion de 111-112°C, probablement non identique à l'acide fusarique, qui a un point de fusion de 108-109°C.

Les symptômes de flétrissement de notre substance sont différents de ceux obtenus à l'aide d'acide fusarique.

Il peut toutefois s'agir dans le cas présent d'un mélange d'acide fusarique et de gibberelline.

En outre nous avons observé, lors du flétrissement des tomates, un brunissement des tiges et quelquefois des vaisseaux tel que le provoque la vasinfuscarine, provenant de *Fusarium lycopersici* Sacc.

L'influence des sources d'hydrates de carbone et de l'azote sur le métabolisme a été examinée. La nature de la source de carbone semble avoir une influence sur la formation des différents toxines.

La carence en ions Cl inhibe la croissance du champignon.

ASPECTS DU PROBLEME POSE PAR LA FUSARIOSE DU DATTIER

par P. PÉREAU-LEROY

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La fusariose du palmier-dattier, anciennement connue sous le nom arabe « bayoud », ravage les palmeraies marocaines depuis plus d'un siècle et s'étend actuellement vers l'est dans les oasis du sud algérien.

L'importance des dégâts est très variable avec les conditions de culture; certaines palmeraies ont perdu, en 30 ans, les 9/10^e de leurs dattiers.

Les essais de traitements, curatifs ou préventifs, n'ont pas, jusqu'à maintenant, donné de résultats satisfaisants, comme c'est le cas général pour les autres grandes trachéomycoses.

D'une étude agronomique et génétique des palmeraies contaminées on peut conclure :

1°) Les dégâts sont d'autant plus graves que la palmeraie est plus abondamment irriguée pendant la saison chaude. Certaines cultures : henné, légumes d'été, Luzerne, Maïs, pratiquées sous les palmiers sont favorables au développement de la fusariose en raison du cycle d'irrigation qu'elles exigent, de la fumure qu'on leur apporte ou de l'enrichissement du sol en azote et en matière organique qu'elles provoquent.

2°) Les variétés de Dattier cultivées présentent une grande différence de sensibilité à la maladie. Certaines, très sensibles, sont en voie de disparition, alors que d'autres sont très résistantes ou même immunes. Parmi celles-ci nous en avons trouvé deux produisant des dattes de bonne qualité.

3°) La vitesse d'évolution de la fusariose est très variable, mais généralement lente; entre l'apparition des premiers symptômes de wilt sur les palmes et la mort de l'arbre il peut s'écouler de 3 semaines à plusieurs

années. De plus, la manifestation du mal sur les palmes est précédée d'une phase d'incubation pouvant, elle aussi, durer plusieurs années. La liaison de ces phénomènes avec les conditions de culture et la sensibilité variétale n'est pas évidente.

Cette lenteur d'évolution constitue, pour le travail de sélection de variétés résistantes, un obstacle important. S'il a été possible de déterminer, assez rapidement, par prospections, le comportement à l'égard de la maladie des variétés cultivées dans les palmeraies où elle sévit depuis longtemps, l'étude de ce caractère pour les variétés introduites des pays non encore contaminés et pour celles qui pourront être créées par hybridation sera longue, à moins qu'une corrélation entre la résistance et un autre caractère génétique puisse être établie. Les recherches dans ce sens n'ont toutefois rien apporté de positif jusqu'à présent.

Après la sélection de variétés résistantes à la fusariose, la régénération des palmeraies dévastées, avec ces variétés, pose d'autres problèmes.

La multiplication végétative du dattier, par rejets, est lente et délicate; il faut rechercher des méthodes permettant la meilleure reprise même avec de petits rejets, c'est-à-dire étudier l'efficacité des substances rhizogènes et déterminer leurs conditions pratiques d'emploi avec ce matériel végétal particulier.

Il semble bien que la fusariose trouve des conditions favorables à son développement dans toute l'aire de culture du dattier. Les possibilités d'adaptation des variétés de cette plante sont moins vastes; et, notamment, presque toutes les variétés résistantes que nous avons trouvées jusqu'à maintenant sont tardives. Il est donc nécessaire, dans le but d'accélérer leur maturation et, de ce fait, d'étendre leur aire de culture, d'entreprendre des recherches sur la xénie et la sélection de palmiers mâles.

THE PSOROSIS DISEASES OF CITRUS THEIR CONTROL OR PREVENTION

by James M. WALLACE

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The different psorosis types described in literature are caused by related virus strains. Although differing in certain symptoms, all strains produce similar effects on young leaves of *Citrus*. Psorosis A, which causes lesions to form on bark of affected trees, is the most common and most destructive of the psorosis diseases. It is world-wide in distribution.

There is no significant natural spread of the virus and seed transmission occurs very rarely. The use of budwood from infected parent trees is the

chief manner by which the disease is increased. Because infected trees commonly show no ill effects and may not develop bark lesions until reaching the age of 15 to 25 years, many diseased trees have been used as sources of budwood. The discovery by Fawcett in 1933, of the leaf symptom of psorosis provided a diagnosis and a means of avoiding diseased trees. A program started in California in 1937 has resulted in the registration of more than 2000 psorosis-free trees for use in propagation. This service has also eliminated hundreds of diseased trees which would have been used had not the inspection service been available.

It is advisable to establish a program for preventing the psorosis diseases in all Citrus-growing regions. Even where the disease at present is not of economic importance it will become so if no protective measures are taken. In California it is well established that the principal *Citrus* varieties, navel and Valencia orange and Eureka and Lisbon lemon, were originally free of psorosis. The disease is quite common in all of them at present. If no control program had been initiated the disease would have increased to such an extent that sources of virus-free budwood would have been difficult to find. This applies also to other *Citrus*-growing regions.

Treatment of psorosis A bark lesions, if done properly, will prolong the productive life of affected trees. Earlier, the lesions were removed by hand with a bark-scraping tool. In California, the chemical treatment with DN 75 (Dow Chemical Company) has largely replaced handscraping. The effect of the treatment is to retard lesion activity and the production of the gum materials which impregnate the xylem tissues and restrict conduction of water.

SPOT ANTHRACNOSES

by A. A. BITANCOURT

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The present check list of the known spot anthracnoses, *i.e.*, plant diseases caused by *Elsinoaceae* (*Elsinoe*, conidial stago *Sphaceloma*), supersedes our previous similar assemblage of them, as of 1936 (cf. Table 1, *Rodriguesia* 2 (número especial: 305-313). It illustrates graphically the many discoveries of new diseases of the group since that date, the total number now far exceeding the 30 or so available for the earlier compilation. Included also are some diseases originally diagnosed in other categories

but now found to be spot anthracnoses. In general these diseases affect many different plants and are of practically world-wide distribution as the new list emphasizes. At the same time it shows that by far the greatest number are found in tropical or subtropical climates.

OUR KNOWLEDGE OF XYLOPOROSIS IN CITRUS

by I. REICHERT

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Xyloporosis disease of citrus was discovered first in Palestine in 1928. Later it was found in Cyprus, Turkey, Brazil, California, Argentine and Florida. The features of the disease as observed in Israel are small and yellowing leaves, early blooming and fruiting, withering and drop of the leaves and general decline. Fruits are frequently assymetrical. Stems, either of rootstocks or scions, show depressions on the outside of the bark, behind which pits, grooves or striations are seen in the wood and corresponding protuberances on the inner side of the bark. Sometimes these symptoms are reverse: tiny needle like protuberances in the wood which fit into corresponding pits in the bark. This form is called by us « Inverse *Xyloporosis* ». The disease may be considered as caused by a virus, although transmission experiments are still lacking in Israel. Evidence supporting this assumption is: 1. Bud perpetuation. 2. Damage of conducting vessels — a symptom of virus disease. 3. High concentration of carbohydrates in upper parts of afflicted trees. 4. Close resemblance to Tristeza, experimentally proved to be a virus. 5. Childs' success in transmitting *Xyloporosis* (Cachexia). Sweet lime stock is mainly susceptible in the above countries when grafted to various sweet orange varieties. In Brazil it is tolerant when grafted to Pera and Caipira, whereas the former combination in Argentine gives rise to the disease. Sour orange stock was found slightly susceptible in Israel and California. Sweet orange stocks show a certain degree of resistance in Israel and Argentine, especially the Valencia in Israel. The following rootstocks were found to be susceptible to the disease in Israel: rough and sour lemon, grapefruit, trifoliata, and to a lesser extent pummelo and sour lime. As scions, the most susceptible were the tangelo Nacotee in Israel and tangelo Orlando in Florida. Less susceptible — the lemons in Brazil. The development of the disease is favoured by prevalence of high temperatures, heavy irrigation and heavy soils. The disease is controlled by early inarching with sour orange. Selected seedlings are less conducive to the development of the disease.

OAK WILT AND ITS CONTROL IN THE UNITED STATES

by A. J. RIKER and J. E. KUNTZ

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Oak wilt, a vascular disease caused by the fungus *Endoconidiophora fagacearum* Bretz (*Chalara quercina* Henry), has spread widely during the past decade among shade and woodland oaks in 18 central and eastern states. All oaks and chestnuts tested have proved susceptible. Red and black oaks die quickly. White and bur oaks, however, often escape infection and, if infected, may persist some years. Following wound infection and rapid fungus distribution in branches and twigs, obstruction of the water-conducting vessels with tyloses and gums precedes leaf wilt, defoliation, and death.

Oak wilt may spread within 15 meters from tree-to-tree through the numerous underground root grafts and over long distances by certain vectors. Sap feeding insects and squirrels may introduce the fungus into wounds. Such spread occurs infrequently and seems quite inefficient.

Where individual trees have great value, the prompt mechanical severing of all root connections has proved effective. Where individual trees have low value, poisoning healthy trees bordering a wilt area has prevented further local spread. The most effective silvicides include sodium arsenite, 2,4,5-T and CMU. The eradication of local centers of infection may reduce or eliminate inoculum for long distance spread.

If present research continues, oak wilt doubtless will be relegated from a menace to merely a nuisance.

A NORTH AMERICAN SPECIES OF
CRONARTIUM DESTRUCTIVE TO *PINUS SYLVESTRIS*

by J. S. BOYCE

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A gall rust damaging to Scotch pine (*Pinus sylvestris*) has been present in the eastern United States and Canada for over 60 years. Long known as the Woodgate rust, recent cross-inoculations prove that the causal fungus is *Cronartium harknessii* native to and prevalent in western North America on *Pinus ponderosa* and other hard pines. Scotch pines of all ages are attacked. Seedlings are usually killed quickly, whereas saplings may live for 20 years or longer but they are generally so malformed as to be worthless.

More than 18,000 globose galls may occur on a single tree. Some trees are resistant, but the basis of such resistance is unknown. Inoculation, which occurs in late May or early June, is directly from pine to pine without any alternate host. Consequently, control is extremely difficult. *C. harknessii* is potentially dangerous to hard pines elsewhere in the world, particularly to Scotch pine in Europe, where its establishment would be catastrophic. Hard pines from North America should not be introduced into Europe except as seed. Inspection of seedlings is valueless because the first symptoms, *i.e.*, swelling of the stem, do not appear until the end of the same growing season in which inoculation occurred. However, in most cases symptoms do not appear until the second growing season, and in some they are delayed until the end of the third season.

LA MALADIE DU « BLEU » DU BANANIER EN GUINÉE FRANÇAISE

par J. BRUN

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Depuis 1937 se manifeste en Guinée française une maladie à symptômes foliaires du bananier désignée sous le nom de « bleu ». Cette altération, aujourd'hui répandue et retrouvée depuis cette époque en basse Côte d'Ivoire, est étudiée à la Station centrale de l'Institut des Fruits et Agrumes de Foulaya (Guinée).

Symptomatologie. — Sur les feuilles adultes, apparition de punctuations diffuses violacées, puis de lignes bientôt confluentes vers la nervure centrale. Cette manifestation est accompagnée ou immédiatement suivie d'un changement de couleur sur l'ensemble de mésophylle, celui-ci devenant jaune orangé. L'altération de la feuille se termine par son dessèchement — quelquefois le bananier se casse au niveau de la base des pétioles.

Les régimes parviennent cependant à maturité mais ne conviennent pas au transport et à la vente.

L'examen des organes souterrains montre une réduction sensible du système racinaire.

Evolution. — L'altération évolue tantôt lentement, tantôt se réduit à des symptômes bénins, tantôt au contraire elle se révèle très brusquement, en quelques jours. Elle se manifeste presque toujours à l'époque de la floraison, soit sur des sujets isolés, soit sur l'ensemble d'une parcelle plantée.

Facteurs de développement. — La maladie intervient chaque fois qu'un déséquilibre de nutrition se fait sentir :

— elle est plus fréquente le long des fossés de drainage où le lessivage du sol est maximum.

— elle n'existe pratiquement pas dans les bananeraies indigènes où les apports d'engrais sont nuls. Par contre elle se manifeste pleinement dans les plantations où un apport massif d'engrais est réalisé. Elle peut disparaître avec la cessation de l'emploi abondant d'engrais.

La maladie n'est pas transmissible par voie végétative ; un rejet atteint du « bleu » peut produire un sujet normal indemne de maladie.

Causes et Remèdes. — Les résultats d'analyses relatives à la teneur en éléments minéraux de l'ensemble des organes du bananier en Guinée : tronc, rhizome, racines, pétioles, limbes, comparés à ceux obtenus dans d'autres régions productrices, révèlent que :

— Le bananier de Guinée souffre d'une carence en Chaux et en Magnésie qui s'exagère par tout apport massif des éléments N.P.K.

— L'apport de l'élément Ca sous forme de Chaux ne procure pas, à lui seul, de modification dans l'importance de symptômes du « bleu ».

— Par contre l'utilisation de Chaux magnésienne à raison de 1 kg par pied, ou de Sulfate de Magnésie (100 gr. par pied en moyenne) dans une plantation où la proportion de « bleu » est de l'ordre de 25.30 %, entraîne, dans la saison qui suit, une régression de l'altération qui n'est plus visible qu'à raison de 1 %.

LUTTE CONTRE *CLITOCYBE TABESCENS*

(FR. EX SCOP.) BRES.

AUTEUR D'UN POURRIDIE DANS LES PLANTATIONS DE *COFFEA CANEPHORA* A MADAGASCAR

par M. H. BARAT

Laboratoire de Pathologie Végétale de Tananarive

Ce *Clitocybe* sévit depuis très longtemps dans la région très humide de la Côte Est. Il a pris récemment une extension sérieuse par suite du vieillissement des plantations. Il s'attaque d'abord aux arbres d'ombrage : *Albizia Lebbeck* Benth. *A stipulata* Boiv. qu'il fait disparaître. Il contamine ensuite les caféiers (Kouilou très sensible, Robusta plus résistant) provoquant la formation de taches vides à extension continue. On observe une diminution du stock d'humus et une augmentation de l'acidité d'hydrolyse. Il y a aussi affaiblissement du caféier par l'insolation à laquelle il n'était pas habitué. Ces divers phénomènes diminuent sa résistance. Cependant le

champignon est très virulent et s'attaque à de belles parcelles notamment dans les terres d'alluvions récentes même les plus riches et les plus favorables à la culture. On le trouve sur les terrains plats ou en pente, siliceux ou argileux. Les engrais et l'installation d'ombrages résistants (*Adenanthera pavonina* Linn.) peuvent avoir une action préventive efficace. Les méthodes classiques de lutte ne sont pas employées par suite de la rareté de la main-d'œuvre. Pour arrêter l'extension des taches avec un minimum de travail, des essais sont tentés à l'aide de sels arsenicaux.

LE DEPERISSEMENT DES ESSENCES FEUILLUES DANS L'EST DU CANADA

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Une affection très grave sévit depuis plus de vingt ans dans les forêts de l'Est du Canada et des parties adjacentes des Etats-Unis. Sur un territoire de plus de 500.000 kilomètres carrés, les bouleaux à papier (*Betula papyrifera* Marsh.) et les bouleaux jaunes (*Betula lutea* Michx.) ont péri parfois assez rapidement ou ont subi une perte de vitalité à des degrés divers. Un dépérissement analogue a été noté chez les frênes dans la Province de Québec dès 1925, puis, un peu plus tard, chez d'autres essences. Cette décadence, observée chez les bouleaux vers 1935 au Nouveau-Brunswick, puis peu après dans les provinces voisines, s'est intensifiée jusqu'en 1950.

La maladie se manifeste par les symptômes de la mort en cime et elle évolue plus ou moins rapidement selon le milieu et les caractères du peuplement. Dans bien des cas, les sujets atteints ont recouvré la santé ou, encore, après une période de regain de vie, ils ont de nouveau dégénéré.

Nos travaux sur cette maladie, pendant une période de près de quinze ans, ont démontré que cette affection n'est pas le résultat de l'attaque d'un insecte du feuillage ni d'un xylophage. De toutes les espèces de champignons trouvées sur ces essences aucune n'a pu être considérée, après essai d'inoculation, comme un pathogène assez agressif pour causer une telle catastrophe.

On a dû aussi abandonner l'espoir de trouver une bactérie ou un virus capable d'engendrer une telle maladie, après des études sur ces groupes d'agents pathogènes effectuées au Canada et aux Etats-Unis. Dès 1940, nous avons émis l'hypothèse que des facteurs physiques du milieu devaient être tenus responsables de ce mal. Cette façon de voir, longtemps controversée, était fondée sur les faits suivants: 1) les symptômes de la mort en

cime chez les bouleaux malades sont les mêmes que ceux observés chez les sujets brusquement isolés par une coupe à blanc-étoc des résineux ; ou des éclaircies par toute autre intervention importante dans le milieu ; 2) dans un peuplement uniforme, le degré de dépérissement est à peu près le même chez la plupart des bouleaux ; 3) la maladie s'est manifestée à peu près simultanément dans un énorme territoire et ne s'est pas disséminée autour d'un foyer central ; 4) les mêmes signes de dépérissement ont apparu à des degrés variables et pendant les mêmes périodes sur d'autres essences feuillues et même sur des conifères.

Outre ces observations générales, confirmées par la suite, nos travaux ont mis en relief d'autres traits particuliers qui consolidaient les notions déjà acquises sur la nature de ce mal. Le comportement même des arbres dépérissants, notamment la nécrose progressive des rameaux et des branches jusqu'à un rameau latéral, qui, selon les circonstances, peut continuer à s'accroître ou mourir à son tour, constitue une indication d'un désordre circulatoire et peut-être plus précisément d'un vice d'absorption. L'analyse des données statistiques a aussi révélé un certain nombre de faits non moins significatifs, notamment : l'intensité des dommages s'accroît avec l'âge des arbres, la maladie est plus grave dans les peuplements ouverts que dans les forêts denses ; sur les pentes orientées au sud les effets de la maladie ont été plus prononcés que sur les versants opposés. Nous avons démontré surtout que le dépérissement est plus intense chez les arbres croissant dans les sols minces et dont l'enracinement est superficiel que chez ceux dont les racines s'enfoncent plus profondément.

Au Nouveau-Brunswick et en Nouvelle-Ecosse, Howbolt et Redmont ont pu montrer subséquemment que la proportion des radicules nécrosées chez les sujets dépérissants est à peu près la même que celle des rameaux desséchés du cimier et que la décadence se manifeste dans le système racinaire avant d'apparaître au sommet de l'arbre.

Ces notions nous ont conduit à l'étude des rapports entre le cours de la maladie et les fluctuations climatiques. Pour évaluer l'état de santé des arbres et suivre les étapes de ce déclin, nous avons adopté la croissance annuelle en diamètre d'un grand nombre d'arbres comme critère le plus commode. Par ce moyen, nous avons pu noter des chutes appréciables de croissance après 1920, 1931, 1937 et 1945. Des courbes de même allure ont été dressées pour d'autres essences que les bouleaux et aussi dans d'autres parties du Canada et des Etats-Unis. En regard des courbes de précipitation pendant les mois d'été, les périodes de chute de croissance ne correspondent pas toujours aux périodes de moindre pluvialité. En revanche, le problème s'éclaire lorsque l'on compare le comportement de la température avec celui des arbres étudiés et un rapport suffisamment étroit entre la température et la maladie a pu être démontré. On ne sait pas

encore si l'on peut dissocier la température de ses effets sur le degré d'humidité du sol notamment dans ce phénomène écologique.

Des études sur le mécanisme intime de ce désordre physiologique en cours au Canada depuis quelques années, ont déjà indiqué que la proportion de radicelles mycorhizées est beaucoup moindre chez ces arbres malades que chez les sujets sains. Un bon nombre de symbiotes fongiques des mycorhizes ont aussi été isolés et un auteur a montré qu'une élévation d'assez faible amplitude de la température peut influencer fortement le développement de ces champignons.

Ces résultats, encore fragmentaires, montrent que des écarts climatiques assez faibles peuvent provoquer des perturbations graves dans le comportement des arbres et se traduire par des catastrophes de grande envergure dans les forêts d'une région.

MERULIUS MOLLUSCUS FR. ANOTHER SPECIES OF MERULIUS FORMING SCLEROTIA

by L. HARMSSEN

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On dug up conifer stumps and rejected boards in damp places a little species of *Merulius*, with an orange coloured fleshy fruitbody which is easily loosened from the wood is sometimes seen. It is *M. molluscus* Fr. (*M. fugas* Fr.). The superficial mycelium consists of threadfine strands, yellowbrown to darkbrown.

During a trip to Norway with the purpose of collecting wood destroying fungi, the author succeeded in isolating some of them. In pure culture the mycelium is light yellow, somewhat transparent and with fine strands, some of which become dark brown in older cultures, bending at rather sharp angles. After 3 to 4 weeks appear in the mycelium small light patches where liquid drops are exuded and rounded sclerotia are formed. At the beginning they are yellowish, later on greybrown with darker spots. They are spherical or more irregularly rounded unto 5-6 mm long. In the agar-medium appear big hollows where sclerotia are formed too. The strands are built up of wide hyphae with somewhat irregularly thickened walls and fine thinwalled hyphae with clamp connections.

Because of the formation of sclerotia in the cultures, the collections were reexamined in consideration of the possible occurrence of sclerotia in the attacked wood. In one collection one sclerotium was found inside the wood, about 1 × 2 mm. It is supposed that the scarce occurrence of sclerotia in the collections means they are formed later in the season. Their formation inside the wood explains also the fact they were not observed before.

Formation of sclerotia in the genus *Merulius* was known before only in the case of *M. pinastri* (Fr.) Burt (*M. sclerotiorum* Falck). The anatomy of the sclerotium is described by FALCK in his: « Hausschwammforschungen » and by MOEBIUS in the paper: « Ueber *Merulius sclerotiorum* ». Both mention the narrow thickwalled longcelled hyphae. In contrast to this the cells in the sclerotia of *M. molluscus* are broad and short, nearly as broad as long and rather thickwalled. By this character the sclerotia are easily distinguished.

As concerns the anatomy, specimens with sclerotia, which earlier were identified as *M. pinastri*, were reinvestigated. One specimen belonged to *M. molluscus*. It came in a board originating from a house, partly decayed by *Coniophora cerebella* too. In the cracks inside the wood were fairly numerous sclerotia, which were flattened down in the narrow cracks. As the board had been taken out in February, no fruitbody was seen, but numerous loose spores indicated an earlier fruiting.

M. molluscus is a rare fungus in Denmark, only 8 localities are known from both frondose and coniferous wood. About its occurrence in buildings, we find little information in literature. ULBRICH mentions one find in Berlin and FINDLAY two finds in London on brickwork in cellars. Nevertheless it ought to be known by those who have to deal with survey of fungi in buildings.

CONCEPTS OF SPECIES AND NOMENCLATURE EVOLVED FROM STUDIES IN THE FUNGUS GENUS *FUSARIUM*

by William C. SNYDER and H. N. HANSEN

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Biological studies of *Hypomyces* (*Fusarium*) *solani*, employing the single spore culture method, reaffirm the relative stability of morphological characteristics in species delimitation over physiological characters. Spore shape proved to be one of the more stable of the morphological features and far more reliable in taxonomy than sclerotium formation or size, spore septation, or spore dimensions. Such physiological criteria as colony pigmentation and configuration, ecologic preference, rate of growth, homothallism or heterothallism, sexuality, and pathogenesis were found unsuited for taxonomic use at the species level. On this morphological basis, *H. solani* is seen to be a large aggregate of biologically diverse but morphologically similar components, with formae defined by their pathogenic capabilities. It is indicated from these studies that taxonomy, in order to serve its purpose

more adequately, should (1) aim to classify fungi at the species level for what they are, not for what they do or where they occur, (2) permit identification to species whether the fungus be living or dead, as long as the appropriate morphological characters be present, (3) not employ pathogenicity nor physiology for species determination and (4) take into account a knowledge of natural variability. Also, an imperfect stage should be considered a suitable basis for the differentiation of a species in a perfect genus but not as the criterion for the establishment of a perfect genus. Furthermore, it is held that international nomenclature would serve better the applied fields if there were fewer and more clearly defined subspecific categories, if only one species epithet were recognized per fungus, if date lines were moved forward to discourage name changing where no taxonomic contribution is made, and if the trinomial system of nomenclature were utilized more consistently to indicate the biologically important features of a morphologically defined species.

THE AGAR PLATE SPORE GERMINATION METHOD FOR TESTING FUNGICIDES

by M. L. GATTANI

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The method described in this paper for evaluating fungicides requires a minimum of equipment and has been found suitable for testing compounds which are soluble in water or insoluble in water but soluble in liquids miscible in water. Its chief advantage, however, is that the day to day variations arising out of the study of germination of spores in water drops is reduced to a marked degree because of the substitution of the conventional water drop by the agar surface.

A NEW APPROACH TO PLANT DISEASE THERAPY

by M. L. GATTANI

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I have based my investigations on the principle that in a vast majority of the fungal diseases of plants which can cause epiphytotics, the secondary infection transmitted by wind is the most important phase of infection. The spores alighting on plant surface germinate and gain entrance in the host

tissue, thus causing infection. If the germination of spores could be prevented on plant surface, freedom from infection can be successfully obtained.

While studying the action of hydrophobic colloidal sulphur (1) on rusts it was observed by me that some uredospores of *Ravenelia sessilis*, a rust very prevalent on *Albizzia lebbek* in Rajasthan, India, did not germinate round about a bacterial colony in one of the control plates. The bacterium was purified by single colony culture and was subsequently identified as belonging to the *Bacillus subtilis* group. Transfers were then made from the purified bacterial colony to one edge of the nutrient agar plates. After four days growth of the bacterium at 23°C, a radial series of four plugs was removed from the agar by a sterile cork borer, the first plug being adjacent to the colony margin. In the mean time, sterile petri plates were poured with sterile 1 percent water agar and the uredospores of the rust were seeded in a circular zone of 1 cm. diameter in the central portion of the plate. To get a uniform distribution of the spores, the agar surface was rubbed with the closed end of a test tube. The four radial plugs were lifted out with the flat end of an inoculation needle and were transplanted in the center of the water agar plates which had been seeded with the uredospores of the rust. The plates were then incubated overnight at 23°C and germination of the spores studied.

The uredospores in the plate containing the radial plug adjacent to the bacterial colony did not show any germination but those in the zones round the radial series plugs no. 2,3 and 4 showed germ tubes of 5, 15 and 50 μ on an average. The size of the germ tubes in the control plates was more than 250 μ . None of the germ tubes showed any lysis, a phenomenon very commonly observed by the author on his studies with the hydrophobic colloidal sulphur. This means that the mechanism of action of the inhibition of germ tubes of the uredospores in this case was different from that of the of the colloidal sulphur.

For field studies, the bacterium was grown in a liquid broth containing molasses 20 gm. and water 1000 ml., pH being adjusted to 7.0. Twenty four hour broth culture of the bacterium was sprayed on the leaves of *Albizzia lebbek* at intervals of two weeks commencing from the second week of August. Three sprays were given and the readings taken in the third week of September. The severity of infection in the controls was almost 100 percent but on the sprayed leaves it was hardly 5 percent. These results indicate that it may be possible to control at least some fungal diseases of plants by spraying them with bacteria inhibiting the germination of the fungal spores.

It may be pointed out here that the bacterium inhibiting the germination of the spores of the fungus must be able to grow well at the temperature which is optimum for the infection of the host plant by the fungus so that when there is a favourable humidity for the germination of the fungal

spores, the bacterium will also show maximum inhibitory activity. A proper selection of the organism and its proper application to the host surface may also lead to a reduction in the frequency of the sprays or dusts, which is a limiting factor in the control of some of the economic plant diseases by the present day fungicides.

ADAPTATION OF FUNGI TO FUNGICIDES AND ITS SIGNIFICANCE IN AGRICULTURE

by M. L. GATTANI

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A species of *Alternaria*, causing seedling blight of wheat, was adapted to increasing concentrations of Agrosan GN and Arasan as a result of successive transfers from media containing increasing amounts of these substances. The adaptability of the fungus was directly correlated with the number of generations for which the fungus had been grown on the media containing Agrosan GN or Arasan. However, when the line adapted on Agrosan GN, was transferred to the Arasan series, it behaved as an unadapted line. The same was true of the line which had been adapted to Arasan. It is suggested that in cases where the use of a fungicide against a fungus becomes ineffective because of the adaptation of that fungus to that fungicide, changing the type of fungicide for the control of that disease would prove very effective.

4. — LES BACTERIOSES DES SOLANÉES

THE BACTERIA PATHOGENIC TO THE SOLANACEAE

by W. J. DOWSON

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RAPPORT

Certain genera of the Solanaceae are subject to disease due to bacteria, one of which attacks so many genera that its specific name is *solanacearum* the cause of the disease known as the Wilt or the Yellowing of the Solanaceae. The bacteria responsible for these diseases are species of five genera, *Erwinia* (or *Bacterium*), *Pseudomonas*, *Xanthomonas*, *Corynebacterium* and *Bacillus*.

The following account includes methods of isolation based on their nutritional requirements and describes the genera and species pathogenic to the Solanaceae. The specific characters by which they may be distinguished are also indicated and in one instance the nature of poisons I have described the symptoms of the disease and suggested its control.

In order to investigate a bacterial disease of plants it is essential to 1) secure the bacteria on suitable media (Starr and Wenz, 1943) from the early stages of the disease, 2) determine whether or not the isolated bacterium is pathogenic, and 3) examine the biochemical activities of the pathogen without delay. If it should be impossible to do this last, the freshly isolated bacteria must be preserved in a suspended state of animation and it can be reactivated for examination. Preservation by freeze — or spit — drying or under mineral (paraffin) oil ensures that the characters of the bacteria remain unchanged either by mutation or adaptation and infection. Cultures many months, or even years, old maintained by periodic transfer will not have the same characters as those of the original isolations, e.g. solanaceous bacteria which produce gas from a sugar when first isolated, often fail to do so after a year's cultivation on artificial media. The ideal way would be to keep them active in their natural hosts, but this is not often practical.

A. GRAM-NEGATIVE BACTERIA

1. *Erwinia* (= *Bacterium*) *carotovora* including *E. arvicola* and *E. atropurpurea*.

This is one of the commonest bacteria, i.e. short rods with a few peritrichous flagella fermenting many « sugars ».

E. carotovora liquefies gelatin and possesses pectic enzymes which enable

it to dissolve the middle lamella of parenchyma to produce a soft-rot. All strains of *E. carotovora* except.

E. aroideae produce gas as well as acid from many carbon compounds including the glucoside salicin. *E. aroideae* produces acid only. *E. carotovora* causes soft-rot of potato (*Solanum tuberosum*), tomato (*Lycopersicum esculentum*) stems and tobacco (*Nicotiana tabacum*) stems and also black-leg of these hosts under humid conditions.

Erwinia carotovora should be isolated on meat-infusion agar and grows well in a simple medium of salts and glucose at 25-30C.

II. *Pseudomonas* species.

These bacteria are sometimes known as the « green-fluorescent bacteria » because they produce the pigment fluorescin in certain media such as meat-infusion agar containing yeast extract (0.05 %). They are long rods with 1 to 7 flagella at one end, i. e. cephalotrichous or polar, but in films often appear in pairs with flagella at both ends (bi-polar). They are not strong fermenters of carbon compounds and do not ferment salicin (21 days) and lactose or maltose only slightly at pH values under 6.8. Therefore, to indicate any small amount of acid which may be produced from lactose or maltose, these sugars should have a pH of 6.8-7.0 and an appropriate indicator such as bromthymol blue, but not bromcresol purple which does not indicate acid until a pH value of 5.5 is reached.

Some species of *Pseudomonas* possess, or are able to make, pectic enzymes and so can produce a soft-rot in parenchyma. Some species liquefy gelatin and some produce acid in purple lactose agar (meat-infusion plus 2 % lactose plus bromcresol purple).

It is thought that the species pathogenic to plants have been derived from two common saprophytes — *Ps. liquefaciens* Migula which liquefies gelatin and *Ps. putida* (Trevisan) Migula which does not.

The following species attack members of the Solanaceae:

1. *Ps. syringae* van Hall which forms a considerable amount of acid in sucrose but very little in lactose and maltose. It has been isolated from rotting potato tubers (Rudd Jones and Dowson, 1950) and in pure culture will produce a yellowish-green soft-rot of potato slices. *Capsicum annuum* and *Lycopersicum esculentum* are also attacked by *Ps. syringae*.

2. *Ps. tomato* (Okabe) Alstatt (= *Ps. punctulans* (Bryan) Nagel, = *Ps. delphinii* (E.F. Smith) Stapp) is the cause of tomato speck and possibly of black spot of delphiniums.

3. *Ps. tabaci* (Wolf and Foster) Stevens (= *Ps. angulata* (Fromme and Murray) Holland, = *Ps. mellea* Johnson) causes the wild fire, angular leaf spot and rust of tobacco. *Ps. angulata* may be regarded as a non-toxin producing strain of *Ps. tabaci* and *Ps. mellea* as a yellow variant also of *Ps. tabaci*. The wild fire bacterium occurs as a saprophyte on the roots of

cereals and grasses in N. America (VALLEAU et al., 1944), hence the appearance of wildfire when tobacco is planted on grassland or after cereals for the first time.

4. *Ps. polycolor* Clara (= *Ps. aeruginosa* (Schroeter) Migula *Ps. pyocyanea* Migula = *Ps. aptata* (Brown and Jamieson) Stevens = *Ps. marginalis* (Brown) Stevens causes a severe leaf spot of tobacco in the Philippines (CLARA, 1930) and is pathogenic to animals. PAINE and BRANFOOT (1924) concluded that *Ps. aptata* (ex sugar beet) was identical with *Ps. marginalis* (ex lettuce) which MEHTA and BERRIDGE (1924) were convinced was the same as *Ps. aeruginosa* (the blue pus organism) ELROD and BRAUN (1942) compared *Ps. aeruginosa* with *Ps. polycolor* and considered them to be identical. Both grew well at 37°C and whereas *Ps. polycolor* was shown to be fatal to laboratory animals, *Ps. aeruginosa* was found to cause typical lesions in tobacco.

5. *Ps. solanacearum* (E. F. Smith) E. F. Smith (= *X. solanacearum* (E. F. Smith) Dowson is the cause of wilting of a large number of solanaceous plants, chief of which are : - tobacco (*Nicotiana tabacum*) (Granville wilt or slime disease), tomato (*Lycopersicum esculentum*) (wilting), potato (*Solanum tuberosum*), egg plant (*S. melongena*), sweet or chillie pepper (*Capsicum annum*). Other genera attacked are : - *Atropa*, *Datura*, *Hyoscyamus*, *Petunia* and *Salpiglossis*.

In addition to wilting and a slimy rot of the cortex of many plants a brown discoloration is formed in the vessels of the xylem in most but not in all and there seems to be more than one variety or strain of this pathogen. Thus in Kenya (NATTRASS, 1945) and in Portugal (MAIA and d'OLIVEIRA, 1945) there is a strain of *Ps. solanacearum* which does not cause the Xylem vessels to turn brown, although it is identical in every other respect with the common strain. SMITH (1914), McCLEAN (1930), da MAIA and d'OLIVEIRA (1945) found that a strain of *Ps. solanacearum* formed acid in litmus milk especially if cream was present. There also appears to be good evidence that biologic strains exist. Investigations by GRIEVE (1936), HOPKINS (1951), McCLEAN (1930) and OKABE (1950) indicate a specific strain in tobacco as isolations from other hosts (peanut, tomato potato) did not infect tobacco.

There is uncertainty about the morphology of this pathogen; it is generally considered to be motile with one polar flagellum, whereas all the strains I have examined appear to be provided with a number of flagella which are not quite polar. Its biochemical activities need more detailed investigation as, for instance, year old cultures ferment salicin a character significant of peritrichous bacteria. The production of acid in salicin incorporated in an inorganic medium and the uncertainty of the number and arrangement of the flagella raises the question of the systematic position of the brown-rot organism. I myself originally included it with *Xanthomonas*

(DOWSON, 1943) but later transferred it to *Pseudomonas* (DOWSON, 1949). Fermentation of salicin and flagella other than polar, if confirmed, would seem to suggest that this pathogen may be an atypical coliform. More detailed information is needed on the nutritional requirements of strains freshly isolated from a number of hosts.

KELMAN (1953) has published an excellent review of the literature concerning *Ps. solanacearum*.

III. *Xanthomonas*. This genus is composed wholly of plant pathogens, each species with one exception being confined to one host genus. All of them, so far, have been isolated from lesions and have been shown to produce similar lesions upon inoculation of the appropriate host plant. None have been found in soil or plant debris except in the immediate vicinity of diseased plants which can be infected from these sources. The cells are slender and oval with usually one polar flagellum though occasionally two may be present. The colonial growth is yellow and copious especially on carbohydrate-containing media. Quite characteristic is the flowing honey-like growth on sterilized potato the starch of which is hydrolysed by most species. Salicin is not fermented within 28 days; most species produce much H_2S from peptone. Litmus milk is rendered alkaline and is digested.

STARR (1946) has shown that whereas most species will grow to some extent in a simple medium such as ammonium chloride, glucose and salts, a few require methionine, glutamic acid or nicotinic acid.

X. vesicatoria, the only species, so far, known to attack members of the Solanaceae, causes a serious spotting of the leaves, stems and fruits of tomato, (*Lycopersicum esculentum*), in sub-tropical countries. *Datura*, *Hyoscyamus*, *Lycium*, *Nicotiana*, *Physalis* and *Solanum* can also be infected. A variety, *raphani*, isolated from radish (*Raphanus*) will also attack tomato and sweet pepper (*Capsicum*), (WHITE, 1930).

X. vesicatoria can overwinter on the roots of wheat, (*Triticum*), (DIACHUN and VALLEAU, 1946).

The pathogen grows well on potato dextrose agar or on meat-infusion-glucose-yeast extract with $CaCO_3$.

B. GRAM-POSITIVE SPORE-FORMING BACTERIA

I. *Corynebacterium*.

The species of this genus are small rods, often club-or wedged-shaped, non-motile forming pale yellow, buff or orange colonies on solid media (agar and potato). When individual cells divide there is a characteristic snapping movement of the two daughter cells which arrange themselves in the shape of crosses, Ys, Ls, or Zs easily seen in a stained film. The species grow very slowly or not at all on ordinary laboratory media but

require one or more vitamins (STARR, 1949) which can be supplied by yeast extract.

A satisfactory medium is potato dextrose agar containing 0.1 % yeast extract buffered with NaH_2PO_4 at exactly pH7.0 (PERRAULT, 1951) on which colonies appear in from 3-4 days at 23°C or a little longer if incubated at room temperature.

1. *C. michiganense* (E. F. Smith) Jensen, is the cause of tomato (*Lycopersicum esculentum*) canker, a seed borne disease. The tomato is the only natural host, although under laboratory conditions species of *Solanum*, but not *tuberosum*, *Nicotiana glutinosa* and *Cyphomandra betacea* can be infected. The pathogen does not survive for long in the soil. Gelatin is slowly liquefied, litmus milk is reduced and digested. The growth on sterilized potato is bright yellow and the potato is darkened.

The pathogen is difficult to isolate from late stages of the disease in which cavities and brown discolouration have developed in the pith of the stem. It can however be readily isolated from the yellow-stained placenta of diseased fruits, from the yellow-stained vascular tissue of young wilting stems and always from the discoloured veins and petioles of leaflets which are wilting.

A good medium for isolation is potato-glucose-agar plus 0.5 % yeast extract.

2. *C. sepedonicum* (Spieckermann and Kotthof) Skaptason and Burkholder, is the cause of the ring-rot disease of potatoes, so serious in the potato-growing areas of North America. The disease also occurs in France and has been recently introduced into Switzerland but was recorded first in Germany (APPEL, 1906). No other plant is attacked in nature but the tomato and egg plant can be artificially infected (LARSON, 1944, LANSADÉ, 1950).

In the growing crop *C. sepedonicum* produces a wilt late in the season when one or more stems are affected; but wilting is not distinctive as it can be produced by other pathogens such as *Verticillium*. To one well acquainted with the disease the early symptoms are quite characteristic. Some of the lower leaves of a stem will appear shiny or glossy and the leaflets soon develop bright yellow blotches and tend to curl upwards with withering margins which turn dark brown. The tuber symptoms are very characteristic and need not be described here, but I would call attention to the fact that all the tubers of a plant are not equally affected; while some show the typical ring-rot, others are so little infected as to appear healthy and yet others have very small patches of pale yellow softened areas in the vicinity of the vascular system.

The sap of the wilting stems and of the yellowing leaflets and also of the small yellow patches just mentioned is full of the bacteria easily seen under the microscope in film preparations made from these tissues.

From such diseased areas *C. sepedonicum* is readily isolated on potato-dextrose-agar containing either 0.1 % yeast extract (PERRAULT, 1951) or 0.1 % asparagine, on which slimy, creamy colonies appear in 4-5 days at 22°-24°C. The pathogen is very difficult to isolate from advanced stages of disease such as the actual ring-rot in the tubers where it is masked by rapidly growing secondary organisms like *Erwinia carotovora* which have gained entrance from the outside through the killed eyes and cracks due to *C. sepedonicum*.

The control of the disease is quite simple and direct and consists in planting whole tubers obtained from a disease-free area. Tubers should never be cut into pieces (sets) for planting as the pathogen is easily spread by the contamination of the knife (PERRAULT, 1948, LANSADE, 1950). They should be graded according to size, the largest for consumption, the smaller ones reserved for planting. It is a significant fact that the disease occurs only in countries where tubers are cut for planting and is unknown in Great Britain where only whole tubers are planted. Furthermore, although the disease first appeared in Germany it has not been recorded from that country since the cutting of tubers for « seed » was given up in 1936.

C. sepedonicum does not liquefy gelatin on the surface of which the growth is yellow as is also that on sterilized potato. There is little change in litmus milk in two months. STARR (1949) has shown that *C. sepedonicum* requires vitamins such as biotin, nicotinic acid and thiamine and also-acids e.g. tryptophane.

3. *C. fascians* (Tilford) Dowson produces leafy galls at soil level on *Nicotiana*, *Schizanthus* and *Petunia* (LACEY, 1939).

Like other species of the genus, *C. fascians* is a slow grower and needs the vitamin thiamine for growth (STARR, 1949). A suitable medium is potato-dextrose agar containing 0.1 % asparagine or 0.1 % yeast extract and also a synthetic medium of NH_4Cl , glucose and salts plus asparagine. *C. fascians* forms a bright orange growth on sterilized potato, turns litmus milk alkaline with a brown rim and orange deposit, and does not liquefy gelatin or only very slowly.

C. GRAM-VARIABLE SPORE-FORMING BACTERIA

1. *Bacillus polymyxa* (Prazmowski) Migula, causes a fruit spot of *Capsicum annum* in Israel (VOLCANI and DOWSON, 1948, VOLCANI, 1949) and also a gummy rot of stored potatoes in Britain (DOWSON, 1943) and in Canada (JACKSON and HENRY, 1946).

Bs. polymyxa is a long rod-shaped motile bacterium with a few peritrichous flagella and forms variable colonies (STANIER et al., 1945).

The spores are large, oval, sub-terminal and wider than the mother cell. Perhaps its most distinctive character is the rapid evolution of gas from sterilized potato within a few hours of inoculation at 37°C. At room temperature pure cultures of *Bs. polymyxa* completely disintegrate slices of potato, carrot, onion cucumber and green tomatoes accompanied by a fruity odour and the formation of gas.

Bs. polymyxa is of some importance in certain industries on account of its biochemical activity and is used to ferment starch to produce butanediol the basis of synthetic rubber. The l-isomer of the diol possesses anti-freeze properties (LEDINGHAM et al., 1945).

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THE CONTROL OF BACTERIAL WILT
(*PSEUDOMONAS SOLANACEARUM*, E.F.SM.)
OF FLUE CURED TOBACCO

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Bacterial wilt (*P. solanacearum*) of tobacco is a serious disease in many tropical areas. It was first reported in the United States of America in 1903. At that time it was confined to a small area in North Carolina. During the following years the disease spread steadily until by 1935 it was widely disseminated and destructive in both North Carolina and Virginia. Crop losses during the 1935-1940 period were about \$ 2,000,000 annually, and the market value of the infested farms was reduced as much as 50 percent.

Research on the host range of this disease has shown that it affects numerous crop plants, as well as many common weeds. Furthermore, the bacteria are able to survive for long periods in certain types of soils. In the United States the disease was especially destructive on certain sandy loam soils that were particularly adapted to the production of fine quality flue-cured tobacco.

Rotation experiments were undertaken with carefully controlled conditions to exclude all possibility of cross contamination. The results showed that if wilt-infested land was planted to wilt-immune crops for one, two, and three years, the tobacco crop that followed was protected against the disease to some degree. However, the reduction in the amount of wilt that was obtained by rotation varied widely from year to year, and consistent, effective protection could not be secured, even after three years of other crops. The frequent failure of rotation to provide growers with effective wilt control had previously been explained on the basis that the growers did not eliminate all susceptible weeds completely. However, it was now proved by carefully controlled experiments that the organism could survive in soil for long periods in complete absence of any host plant. After three years of bare fallow, 68 percent of the tobacco developed wilt, and after four years of the bare fallow there was still a loss of 27 percent due to wilt. These experiments also showed that more infection survived in the bare fallow plots than in the corresponding plots which were cropped to soy beans or corn. The wilt eliminating efficiency of corn, completely free of all weeds, was compared with corn plus a scattering of susceptible weeds. These comparisons were continued over a five-year period and at the end the mean value for wilt infection after weed-free corn was 36.2 percent, and after weedy corn 32.4 percent. Thus the

failure of tobacco growers to effectively control wilt by rotation was not due to the fact that they did not completely eliminate weeds. It was concluded from the rotation studies that even a four-year rotation could not be depended on to control wilt, and longer rotations than this were not practical, due to the rather limited areas of land suitable for tobacco culture on the average farm.

Chemical soil treatment studies resulted in the discovery that comparatively heavy applications of urea, 1,000 pounds per acre, would control wilt quite effectively. Furthermore, it was possible to apply the urea in the fall, grow a corn crop the following year, to use the excess nitrogen, and then produce a successful crop of good quality flue-cured tobacco. The urea treatment has been used to some extent in the Florida shade tobacco area, but it has not been adopted in the flue-cured area because disease resistance has proven to be a more economical means of controlling wilt.

Search for wilt resistance was initiated on a systematic basis in 1934, and special collectors covered the entire area to which *Nicotiana tabacum* is considered native-Mexico, Central America, and all of northern South America. The collections were evaluated for wilt resistance; first, in the greenhouse to eliminate all that were completely susceptible, and then those showing some degree of resistance were subjected to severe field tests at a special location near Creedmoor, North Carolina, in the locality where wilt was first found in 1903. A total of 1,034 collections of *N. tabacum* were tested in the greenhouse and 129 of these were subjected to subsequent field tests at Creedmoor. Out of all this material, one greenhouse selection, T.I. 448A, made in a collection for Colombia, South America, showed uniform high resistance to wilt in the subsequent field tests. Highlevel wilt resistance has not been found in any other collection of *N. tabacum*. Furthermore, evaluation of numerous *Nicotiana* species has failed to reveal appreciable wilt resistance in any of these. Most of the species, in fact, were more susceptible to bacterial wilt than was *N. tabacum*.

T.I. 448A was crossed with various flue-cured varieties and numerous F_2 selections were made. Field evaluation for resistance, plus cured leaf evaluation for quality, showed that the cross of 448A with the variety 400 was most promising. Further selection and testing resulted in Oxford 26. This variety, intended originally to be merely a temporary introduction to meet an immediate need, later proved to be very desirable with respect to quality. It has now been largely superseded by subsequent introductions, Dixie Bright 27 and 28, which surpass Oxford 26 in yield.

The inheritance of the wilt resistance found in T.I. 448A is complex. By using very large populations it was possible to recover in the variety, Oxford 26, practically the full resistance of T.I. 448A. To accomplish

this, however, it was necessary to self and select through the F_3 generation before the full resistance was recovered and stabilized.

The desirability of combining bacterial wilt and black shank resistance was early recognized. Since resistance to both diseases is inherited on a polygenic basis, it was feared that combined resistance, plus suitable plant type, yield, and quality, might be difficult to secure. However, evidence was soon obtained of some linkage between the genes for wilt and black shank resistance. Crosses involving the two readily yielded segregates with some resistance to both diseases. Large scale evaluation of the lines selected from these crosses, along with further selection, has since produced a very successful commercial variety, Dixie Bright 101, with good resistance to wilt and moderate resistance to black shank. At the present time, 1954, due to the use of wilt, and wilt-black shank resistant varieties, the damage caused by wilt in the United States has been largely eliminated.

THE *L.* FORMS OF BACTERIA

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The now outmoded dogma of the monomorphism of bacteria has kept us from realizing that naked masses of bacteria, the *L.* (KLIENEBERGER) *forms*, exist and that brown root lesions, gum-like masses in vessels and other so-called « host reaction phenomena » are nothing but parasitizing and ageing masses of *L. forms*, pigmented and coagulating because of their own products of metabolism, supposedly flavins, insoluble in sap and cytoplasm. The *L. forms* may be seed-borne, attack meristems, feed on growth substances and are inactivated by trace elements. Quite possibly the application of inorganic fertilizers may transform soil-inhabiting bacteria or those of the root episphere to phytopathogenic *L. forms*.

The whole problem seems to reveal a frightening abyss of ignorance.

5. — *LES BACTERIES DES FRUITS TROPICAUX*
**BACTERIAL DISEASES OF ECONOMIC PLANTS
IN CALIFORNIA**

by Peter A. ARK

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RAPPORT

Bacterial diseases of plants are found in every part of the world wherever plants are grown. Their economic importance is determined by the inherent capabilities of the bacteria causing them and by the value placed on the plants subject to attacks by such bacteria.

The destructive powers of plant pathogenic bacteria in addition to inherent toxins etc. depend largely on climate, cultural practices and the varieties of plants subject to the attack. California possesses a wide variety of climates, crops and soils and thus becomes a fertile field for numerous bacterial plant pathogens which are found in both the tropical and temperate zones of the globe. In the principal agricultural areas, however, most of the rainfall is rather sharply limited to the winter months, November to March, when perennial plants are dormant or quiescent. This, with the comparatively mild winter temperatures, favors the initiation and progress of such diseases as bacterial gummosis of stone fruits, blast of apple, citrus and pear, and olive knot. The rains may overlap the beginning or the end of the growing season sufficiently to influence profoundly the course of disease as in the case of fireblight and blossom blast of pear and bacterial gummosis of stone fruits. The dry summers on the other hand tend to depress such above ground diseases as the bacterial blight of bean and angular leaf spot of cotton and this fact is used to advantage in the production of disease free seed. Citrus canker and bacterial spot of peach are unknown in this area though they have no doubt been introduced more than once.

Bacterial diseases may be classified, as are diseases caused by other types of pathogens, into necroses and tumefactions and subdivided under necroses into blights, blasts, rots, and leaf spots.

Of course, it is impossible to give a complete picture of the occurrence of bacterial diseases of plants in California because of the multitude of crops grown in the state. The present discussion will be confined to a few diseases and primarily those which are of considerable economic importance and which will illustrate pathologic as well as control aspects of the disease. A synoptic presentation of the pathology of the disease will be followed by development of a concept of successful control of the disease when feasible.

BLIGHT DISEASES

There are two major bacterial blight diseases in California. They are important because of large acreages involved in production of each crop and also because of severity of the damage brought on by the diseases. The first is fireblight of pears and related species and the second is walnut blight. According to the latest statistics on the acreages of pears and walnuts, there were, in the state of California, 39,340 acres of bearing pears, with the approximate tonnage of 386,000 tons and \$ 20,000,000 of production value, and 112,632 acres of bearing walnuts, with the approximate tonnage of 73,000 tons valued at \$ 30,441,000. Most acreages are situated in areas favorable for the development of the above mentioned diseases.

Fireblight. The disease is most noticeable, in the spring of the year, on blossoms which, when attacked by the disease, turn dark brown and then black and, as the disease progresses, it involves fruit spurs, twigs, branches, the trunk, and the root. More detailed progress of events in the fireblight is as follows. In the spring of the year, if there is enough moisture in the air or if it rains, the nectar becomes diluted to the extent that the sugar concentration is brought down to 2 percent or less. This low concentration of sugar together with other ingredients of the nectar constitutes a very suitable medium for the fireblight bacteria. Under favorable conditions, the activity of the bacteria can be swift and quite spectacular. Toxic products of the physiologic activity of the bacteria kill the cells of the nectary and receptacle and thus the bacteria enter the host and from here proceeds internally to other parts of the plant. Thus the entrance of the bacteria is effected through the most ideal combination of climate and nutrition of the pathogen. Thence the bacteria travel through the bark of the pear, downward and upward, killing smaller or larger areas on the plant depending on temperature, humidity and the status of the host. Extensive cankers can be formed on a tree carrying the bacteria through the winter. The cankers are dormant in the winter but wake up in the spring at which time masses of the bacteria are liberated to the surface, in the form of exudate, commonly known as a bacterial ooze. This exudate has a particular appeal to various flies, ants and other insects. As a result of their contact with this infectious material, the disease is introduced into the blossoms and the cycle is repeated. Dissemination from blossom to blossom is accomplished by bees, flies, and other insects. Cankers represent the most important reservoir of infection and must be eliminated as a source of the disease. The interior of the affected bark at first appears only slightly water-soaked, later becoming reddish-brown, changing eventually to dark brown or black. Holdover cankers are the main sources of fireblight in pear orchards.

Walnut blight. Caused by a yellow bacterium, *Xanthomonas juglandis*, walnut blight is the most destructive disease of the English walnut in California. It occurs in areas having a high humidity in the spring and early summer. In 1952, the loss in the walnut crop from this disease was estimated at \$ 2,000,000. In severely affected orchards, the disease is known to affect as high as 98 percent of the nuts. The disease does not kill the tree and thus would have not been of economic importance if it were not for destruction of the nuts. All young parts of the tree are affected with the exception of the roots. On the leaves the disease appears first along the margins of the leaflets as small dark spots, water-soaked with yellowish-green halos around them. The spots can cause disfiguring of the leaf and sickle-shaped leaves can be seen in large numbers in case of a heavy attack. The lesions occur eventually on all parts of the leaf. Since the disease is primarily of rapidly growing tissues, all young tissues are subject to the attack by the organism early in the season. With hardening of the tissues on the shoot the disease slows down and later is arrested completely. The older tissues of the plant are not susceptible to the disease. The shoots that are affected by the blight have a dark brown or black appearance and can be killed to a distance of 6 inches or longer. The lesions may run deep into the pith. Under favorable conditions in the orchard, small but numerous cankers can overwinter and serve as a source of primary inoculum. In most cases the organism dies out at the end of the second season.

Male flower buds, known as catkins, can be attacked while in the semi-dormant state and upon further expanding killing takes place. Individual florets are invaded and the pollen is frequently contaminated by bacteria. These pollen grains are carried by wind to all parts of the tree and to other trees and settle down on the pistillate flowers where they cause the disease. Walnut blight is primarily a disease of parenchyma although other tissues may be involved at later stages of development. The other main infection courts in this disease are the stomates. Considering the overall picture of walnut blight it can be said that under California conditions the reservoir of infection is in overwintering blighted shoots carrying numerous cankers, and in blighted leaf and flower buds.

BLAST DISEASES

Blast diseases are caused by various strains of *Pseudomonas syringae*. *Pear blast*. Pear blast occurs on blossoms, twigs, cluster bases, branches and trunks. Its severity is due chiefly to the fact that a complete loss of the crop may result if favorable conditions for the occurrence of the disease prevail during blossom time. The severity of the disease is frequently correlated with cold, wet weather prevailing at the time of flowering. In contrast

to fireblight it is a disease of cool climates. As in the case of the fireblight, blast starts in the blossoms killing them rapidly. Killed blossoms appear very black and never form exudate, or ooze. With the arrival of fair weather killed blossoms drop off very quickly. From the blossoms the disease moves into cluster bases and from there into shoots but not usually very far. Cankers may be formed around lenticels and pruning wounds on older branches and the trunk but are usually limited to the outer bark. The disease on cluster bases and small branches is characterized by the spongy condition of the cortex and outer phloem. On some varieties of pear there can be observed sloughing away of the periderm which is at this stage of a tan color. Dormant blossom and leaf buds can be killed by the disease. The disease is active during fall, winter and early spring. Oversummering and overwintering takes place in numerous cankers of the previous season as well as in dormant leaf buds. Dissemination of the pathogen is by rain.

In passing it can be stated that the pear blast organism, *Pseudomonas syringae*, can be transmitted to citrus trees. In this connection observation of Dr E. E. Wilson, of the University of California, is significant. He stated that « in 1932, within two weeks after numerous new cankers appeared in an orchard of Wilder pears, typical citrus blast appeared in a row of orange trees bordering the orchard. No blast occurred on these orange trees within the preceeding three of four years, and close examination after the outbreak in 1932 failed to show any recognizable blast symptoms of previous years ».

Bacterial gummosis of stone fruits. Bacterial gummosis of stone fruits is a very destructive form of blast occurring on many kinds of stone fruits. It is a most important bacterial disease of plums, cherries, peaches, almonds and apricots in California. It is widely distributed in the state where 229,276 acres of stone fruits and 91,993 acres of almonds are being grown with a total production value of \$ 119,515,000. In many respects it resembles pear blast. However, it is different from the pear blast in that oftentimes extensive and deep-seated branch cankers are formed which constitute the most destructive phase of the disease. The bacteria attack leaves, blossoms, fruit, pedicels, green shoots and the buds. Cankers result from bud infections. Sweet cherries develop leaf and blossom infections more frequently than other stone fruits. Of early varieties of plums, the more susceptible kinds are Duarte, Santa Rosa and Kelsey, while among late varieties the most susceptible ones are represented by Grand Duke, President and Tragedy. Overwintering of the organism takes place in winter buds and cankers. Activity of the disease begins in late autumn, continuing through winter and spring. It is halted during the summer. Some evidence points to a direct relation between the growth processes of the tree and the cessation of the canker extension.

Citrus blast. Citrus blast is an economically important disease in the

Northern part of California and is especially severe on naval oranges. Leaves and young shoots are the principal parts affected by the disease. Serious defoliation and dieback reduce the vigor of the plant and undermine the fruit production. The disease is favored by strong winds, low temperature and heavy rainfall during early winter and spring. Although the wounds made on the leaves by strong winds are important portals of entry, there is evidence of stomatal infections on the leaves and young shoots. The trouble is essentially a parenchyma disease. The lignified tissues of the stem are not invaded and the organism is not found in the vascular system of the plant. Fruit becomes infected by the organism through wounds made by the thorns which often have small lesions right on the sharp point. The organism survives in small cankers of the previous year at the base of the thorns and active bacteria are not found in older cankers.

TUMEFACCTIONS (GALLS)

Tumefactions or galls are hyperplastic diseases in which the causal organism enters through natural openings or artificial wounds and produces extensive proliferation of the cells. There are three diseases of this type that are causing considerable losses, namely olive knot, oleander knot and crown gall.

Olive knot. Olive knot is widely spread in many olive groves in California and especially in those grown in the Northern region of the state. Sevanillo, Nevadillo Blanco, Manzanillo and Ascolano are the most susceptible varieties, although Mission olive can be heavily infected in some years. One of the reasons why Sevanillo has olive knot more severely in the trunk area than the Mission olive is because of the presence of more dormant buds on the trunk in this variety than in the Mission. The disease can be spread from tree to tree but not by the agency of flies or other insects as is the case in Mediterranean regions but by rain and wind. The bacterium which incites the disease, *Pseudomonas savastanoi*, is a wound parasite and does not infect the host without open wounds. Bacteria are present in great masses in active tubercles (knots) and are exuded in profusion when the knots are wet. Most infections on a tree are through leaf scars which are receptive to the organism for about one week after the leaf falls. At the end of one week phelloderm is formed and the scar is not susceptible to the bacterium any longer. The blossom scars form the phelloderm about 13 days after abscission. That leaf scars are very important portals of entry is shown by the fact that current, or one-year-old branches are seldom attacked, even though they arise from badly diseased limbs. When the disease appears on one-year-old growth, it is usually located at leaf scars or at blossom or raceme scars. Next to the leaf, blossom or raceme scars, come growth cracks present in large numbers on trunks as a result of suckers

arising from the trunks. The disease is active in December, January and February, but sometimes this period is extended to April and May.

Oleander knot. Oleander knot is in many respects similar to the olive knot and is caused by *Pseudomonas tonneliana* which is almost identical with *Pseudomonas savastanoi*. Abundant knots are formed on stems at the scars made by falling of the leaves and considerable distortion of racemes and seed pods results in epidemic years. The disease reached epidemic proportions in many plantings of oleanders in California for the last few years due to heavy rainfall and the presence of huge amounts of infection on the shrubs. The knots were also in evidence on the leaves due to wounds made by rubbing against the stems and against each other during strong winds. Heavily infected shoots may die but the plants are not known to be killed by the disease. Badly diseased plants show scanty foliage and limited growth.

Crown gall. Crown gall, under California conditions, attacks mainly stone fruits, walnuts and brambles. Occasionally it is found in abundance in hop fields, vineyards and roses. Other species of plants do not seem to be seriously attacked by the crown gall organism, *Agrobacterium tumefaciens*, especially in acid soils. The disease is confined to underground parts and trunks of susceptible plants. Sometimes aerial galls can be found in greenhouse grown roses. The pathogen enters the plants through injuries inflicted by agricultural implements and only occasionally by insects as in the case of the brambles and the hops.

SOFT ROT

Bacterial ring rot of potatoes. Ring rot of potatoes is an example of the soft rot disease of only potential importance in California at the present time. It was very serious about twenty years ago but due to the use of certified seed potatoes and sanitary measures, it now occurs occasionally and in situations where non-certified seed is used. The disease is caused by *Corynebacterium sepedonica* and is extremely infectious. It is most conspicuous in the tubers. On above ground parts of the plant it is recognized by the yellowing and marginal wilting of the leaves which may drop off late in the season. Necrotic spots can be seen between the veins of the leaf. Affected vines gradually turn yellow and dark brown when the disease invades the whole plant. Both vascular and storage tissues of the tuber are invaded by the pathogen. On cutting the tuber, in mild cases, a pale yellow color can be observed in the vascular ring. As the disease advances there can be seen a separation of the vascular ring from the cortex. At this time the tissues are mealy in appearance and are easily separated on pressure or by prying with a knife. Cavities are formed along the vascular ring. The disease is perpetuated through the use of infected potatoes for seed. The

cutting knife is capable of carrying infection from one cut to another. Experimental evidence shows that a knife used for one cut through a diseased tuber will carry the pathogen to twenty seed pieces of healthy tubers. The bacterium is resistant to desiccation and is known to live for nine months on metal surfaces in unheated field barns.

LEAF SPOTS

True bacterial leaf spots of plants are not of frequent occurrence in the open, in California, due to its climate devoid of summer rains. The occurrence of the true leaf spots is largely confined to the crops grown in greenhouses or lath-houses.

Gardenia leaf spot. Leaf spots on gardenia are caused by *Xanthomonas maculifolium-gardiniae*, a yellow bacterium. The disease starts on tender leaves as minute dots gradually enlarging, showing at first a pale yellow center, which later becomes reddish-brown, surrounded by a yellow halo. The margins of the lesion are somewhat thickened and have a greasy appearance. Several spots may coalesce to form a larger one. The disease causes a premature abscission which leads to lowered vigor of the plant with subsequent lowering of production of flowers. The disease is spread from plant to plant by syringing to reduce the red spider population. When gardenias are forced for flower production, the high humidity and air temperature usually maintained provide excellent conditions for the spread of the organism and subsequent development of epidemics. The organism enters the leaves through stomatal openings.

Leaf spot of tuberous begonia. Tuberous begonia grown in the lath-house develop bacterial spots due to *Xanthomonas begoniae*. The symptoms of the disease consist at first of small, circular, blister-like spots, which later become very conspicuous and transparent. Gradually the spots enlarge and then coalesce, forming large, blister-like, necrotic lesions. These lesions appear water-soaked, while occasionally a yellow, dry ooze may be observed on dry specimens. The lesions are often very numerous and cause premature abscission of the leaf. Invasion of the vascular system of tuberous begonia plants was observed only in the greenhouse after spraying them with a heavy water suspension of the organism in a moist cabinet. After 8 to 12 days, the plants were transferred to a warm greenhouse. This phase was characterized by a water-soaked appearance of the petioles, which slowly spread into the main stem with a gradual softening of all tissue. Subsequently, the entire plant collapsed.

CONTROL OF BACTERIAL DISEASES OF PLANTS

Control of bacterial diseases of plants has not yet reached the degree of perfection attained for certain fungous diseases, such as apple and pear scab or leaf curl of peach. In many cases of bacterial disorders, the diffi-

culty consists in the ability of plant pathogenic bacteria to establish a degree of protection in the plant which makes the access to them a very difficult task. Thus, in the case of pear or apple blight, the causal agent, *Erwinia amylovora*, oversummers and overwinters in the bark and cannot be easily reached by the use of even the best known bactericides. The area occupied by the fireblight cankers sometimes is so great that application of chemicals to destroy the cankers may lead to fatal injury to the plant. In cases where the lesions are not too extensive, a combination of surgery and drenching with zinc chloride or mercury salts in alcoholglycerin solution will help in eliminating the reservoir of infection. In blast diseases the situation in regard to the reservoir of infection may be even more complicated because of the numerous foci of infection. As in the case of the bacterial gummosis of stone fruit, bacteria may be found in cankers, dormant leaf and fruit buds. Cankers on affected trees may run from top to bottom of the tree and not in a straight line. It is futile to attempt to reduce the bacterial potential by local treatment of such cankers. In pear blast the sources of bacteria are located in blasted fruit buds, leaf buds, small and large cankers on twigs, branches and trunk. An analogous situation is present in citrus blast where diseased areas are abundant and often inaccessible for physical removal.

The nature of the behavior of pathogenic bacteria on the plant is such that control measures must be based on the knowledge of the important courts of entry of the bacteria into a plant and stopping the pathogen at that point, leaving the question of the reservoir to be dealt with separately, i.e. efforts have to be directed to prevention of the disease from establishing itself on new parts of the host. This approach to establishing effective control of plant diseases of bacterial origin is best illustrated by controlling fireblight in pears under California conditions. It has been mentioned earlier in this paper that the initiation of fireblight takes place in the blossom where the nectar furnishes sugar and other nutrients for the bacteria. If a good and safe bactericide is introduced into the blossoms, it is capable of inactivating the bacteria as they arrive there thus preventing the disease from becoming established. Barring the bacteria from initiating the disease in the blossoms leads to the absence of the disease on twigs and thereby prevents formation of the reservoir of infection in the form of cankers. When this principle of control of fireblight is employed, the disease becomes manageable by virtue of the fact that old cankers can be taken care of by surgical means leading to eventual elimination of the disease from the orchard.

For a number of years California pear growers have practiced so-called copper schedule in handling the fireblight. It consists in applying copper either as a weak Bordeaux or as a dust, such as copper sulfate lime mixture, at the blossom time, beginning with about 20 percent open bloom and

continuing it until no more bloom appears. Spraying or dusting is done either just prior to or immediately after rains in so far as feasible. The number of applications varies from 4 to 11 or more, depending on the weather. Perfect control can be obtained if the dust or spray is carried out with due consideration to blooming and weather. It must be mentioned that although copper is a good bactericide, it produces, under some climatic conditions, some injury on pear fruit known as russetting. This russetting makes the pear fruit less attractive to the consumer and it commands a lower price in the market.

In this respect, the experiment of 1953 on the use of streptomycin to control pear blight is suggestive of possible substitution of this antibiotic for copper sprays and dusts. Bentonite dust, containing 240 p.p.m. of streptomycin, was dusted on 25-year old Bartlett pears during the blooming period and the control of fireblight obtained by this method was comparable to that with the copper-lime dust and with no visible russet. The experiments in Ohio and Missouri on use of streptomycin spray containing 100 p.p.m. gave good control of apple blight in 1953. Thus, it appears that prevention of the disease at the infection court is possible when full recognition is given to the ecological aspects to the disease.

The application of this principle is also demonstrated in the partial control of the olive knot disease. It has been established that in this disease the infection court is ordinarily confined to leaf scars made by abscission of the leaves. This infection usually takes place in the fall of the year and may continue during winter or even into the spring. Application of home-made Bordeaux 4-4-50, in fall, winter and spring reduces the incidence of the disease. Again a certain amount of injury is encountered at times but not serious enough to preclude the use of the copper. It is possible that in the future substitutes can be found for the copper.

In citrus blast, infection courts are numerous and are in the form of wounds made on the leaves due to strong winds not to mention stomatal infections. The disease becomes active in November, coinciding with rain, wind and heavy fogs. Copper sprays applied, beginning in September and continued through November, showed a considerable reduction in the disease, in the experiments performed in 1953.

In the crown gall disease, infection courts are primarily man, insect and naturally made wounds. In this case disease can be controlled by practicing careful cultivation or applying a disinfectant such as calomel in case of peach seed treatment. The reservoir of infection present on the plant can be eliminated by treating individual galls with such a preparation as 20 % elgetol-methanol drench.

The principle of controlling the disease at infection courts is also demonstrated in handling bacterial ring rot of potatoes, *Corynebacterium sepedonica*. As is known to all who worked with the disease, it is a highly infectious disease. A cutting knife is capable of carrying the bacteria

to many pieces of potatoes if not disinfected. The fresh wound of the potato is an important infection court. However, if the knife is properly disinfected the disease can be confined only to pieces of potatoes which carried the disease before being cut. A perfect control of the disease is attained by eliminating the disease in the seed. Potato seed certification with zero tolerance for the ring rot resulted in complete elimination of the disease in most potato fields in California.

In leaf spot diseases, infection courts are represented by the stomates. Protection of the stomates either by use of chemicals or by keeping the surfaces dry will prevent the disease in many cases. Although under field conditions it is often impossible to fulfill the last mentioned requirement, it is a practical possibility for some greenhouse crops. The use of furrow irrigation as against overhead watering avoids the angular leaf spot of cotton, *Xanthomonas malvacearum*, in the San Joaquin valley, in California, where the summers are rainless. A striking example of controlling, by keeping leaf surfaces dry, a serious leaf spot disease of greenhouse grown gardenia, caused by *Xanthomonas maculifolium-gardiniae*, can be mentioned here. In one of the gardenia establishments near San Francisco, California, this gardenia leaf spot was present in epidemic form due largely to frequent syringing for the red spider mites. Copper sprays were of little use because of the continuous removal of the chemical by washing. With the introduction of Azobenzene 70 fumigant for the control of red spiders, the disease diminished noticeably right from the start of the fumigation practice and now this disease does not exist in that establishment.

Elimination of a leaf spotting disease of beans caused by *Pseudomonas phaseolicola*, by chemical means is demonstrated by application of streptomycin spray in concentration of 100 p.p.m. as was recently shown by Dr. Mitchell, of U.S.D.A.

The discussion is not complete without mentioning the possibility of finding chemicals that can be used systemically to prevent bacterial diseases of plants. Work with streptomycin tends to indicate that this antibiotic is capable of penetrating plant tissues in concentrations sufficient to prevent pathogenic bacteria from initiating a disease. In our greenhouse experiments on control of fireblight in *Pyracantha angustifolia*, it was clearly indicated that the amount of the blight in shoots was in proportion to the number of daily applications of streptomycine sulfate. Thus, in terms of the average linear inches of the blighted parts per shoot, there was 5.9 inches in the untreated check, 5.5 inches with a single application of the streptomycin, 4.6 inches with two consecutive sprayings, 3.5 inches with three, 2.8 inches with three, 2.8 inches with four, 2.3 inches with five, and 1.9 inches with seven consecutive daily applications of the spray. A suggestion of the movement of the streptomycin within the plant is contained in Mitchell's experiments with halo blight of beans caused by *Pseudomonas phaseolicola*.

By painting a portion of the stem of the bean plant he was able to demonstrate the movement of streptomycin and dehydrostreptomycin inside the plant upward into the upper leaves in a concentration sufficient to prevent the development of the halo blight following inoculation with a virulent culture of the pathogen. No accumulation was noted in the seed following strong application to the stem. The accumulation of the streptomycin in the treated parts was observed up to three days after which the level gradually dropped to nothing. The movement of the streptomycin in the plant was upward but not downward. It must be pointed out that this indication of translocation in the plant is, so far, shown by streptomycin only. Other antibiotics tested on the bean, such as neomycin, bacitracin, polymyxin, tyrothricin, subtilin, penicillin G, terramycin and aureomycin, showed no tendency to migrate in the plant or protect the plant from the disease.

In conclusion, it can be said that it appears that through knowledge of infection courts and relationships between plants, climate and bacteria, many bacterial diseases of plants can be controlled satisfactorily. The introduction of antibiotics into phytopathological work may lead to improvements in chemical treatment of bacterial diseases of plants by affording chemical agents with less phytotoxicity and capacity to protect the plants systemically.

ON THE TROPICAL ELEMENT OF PLANT DISEASES

by I. REICHERT

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A new approach to plant pathology was some time ago put forward by us (*Proc. Intern. Bot. Congress*, Stockholm, 1950; *Riassunti Congresso Intern. Microbiologia*, Roma, 1953). It consisted of using biogeographical methods in the classification of plant diseases in groups characterized by various biological aspects such as distribution, ecology and origin. It was shown that such an analysis enables the prediction of the appearance of plant diseases to be made. The group embracing all the diseases originating from the same area was designed as an « Element ». An attempt is here being made to discuss the tropical Element of plant diseases *i.e.* of those which derive from tropical countries, characterized by high temperature and high air humidity. Plant diseases can only be allotted to the tropical Element when they respond to the two following conditions: 1) They must have their centre of distribution in the tropics; 2) Their ecology has to conform with the nature of the tropical climate. With this definition in mind a number of plant diseases occurring in Israel (characteristic also to other countries with a similar climate) was chosen for the purpose of examining whether they may be classified as belonging to the tropical Element. The pathogens to be dealt with are: 1) *Bacterium solanacearum*; 2) *Phytophthora parasitica*; 3) *Diplodia natalensis*; 4) *Fusarium bulbigenum*; 5) *Sclerotium Rolfsii*; 6) *Sclerotium bataticola*. All these pathogens are common in the tropics and in humid warm countries such as south east of U.S. and Asia. They all require high humidity and temperature for their development. Their minimum temperature is not less than 8-10°C, their optimum range varies between 30-35°C and their maximum may reach 37-40°C. From the above description we see that these organisms conform with the prerequisites required for their characterisation as a tropical Element.

We have now only to deal with the question how is it possible that the tropical Element with its high exigence to humidity could acclimatize itself in a xerothermic region such as the eastern Mediterranean and the pacific south west of U.S.A. Elsewhere we have shown (*Inst. of Biology*, London 1952) that these areas do not permit the entrance of hygrophyllic plant pathogens. The explanation of this phenomenon is; 1) The abundance of humidity, due to irrigation, in the lower parts of plants vulnerable to infection; 2) The development of some of these diseases (*B. solanacearum* and *Ph. parasitica*) only in humid spring time; 3) Their occurrence within the hosts and thus being protected against direct danger of air drought during the dry summer time. No leaf diseases deriving from the tropics occur in these xerothermic regions. These facts have to be taken into consideration when carrying out a phytopathogeographical analysis.

7. — *INTERFERENCES ENTRE VIRUS*

INTERFÉRENCES OF VIRUSES

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RAPPORT

With reference to recent comprehensive summaries of this question by BENNETT (1951, 1953) a more schematic report of the problems might be preferable.

Interferences between plant viruses can become manifested as

1. the protection designated as « immunization », « cros-protection », « premunity » (QUANJER, 1946). Strains of most mosaic and ringspot viruses give high degrees of mutual protection.

2. the suppression of one virus by another, e. g. potato-virus Y and *Hyoscyamus-virus* 3 by severe tobacco etch-virus in tobacco (BAWDEN and KASSANIS, 1945).

3. a regulated equilibrium in multiplication of the component viruses, e. g. some mosaic viruses in tobacco (THUNG, 1937) and tulip breaking viruses (McWHORTER, 1938).

4. the formation of a virus complex which induces disease symptoms of a new type, e. g. tobacco rosette (SMITH, 1946).

5. a synergism in favour of one of the viruses, e. g. potato virus X by potato virus Y in Turkish tobacco (ROSE, ROCHOW and SIEGEL, 1952).

Besides these cases of interferences it is known that the plant can simultaneously carry two or more viruses which are multiplying independently, e. g. viruses of curly top, yellows and mosaics in sugar beet (BENNETT, 1953).

Since virus multiplication is the result of interaction between the infecting particle and the living cell, one may say three types of relationships determine the interferences between two viruses:

- a) the relationship of the first virus to the host cell;
- b) the relationship of this virus to the second one;
- c) the relationship of this second virus to the host cell as changed by the first virus.

This group of relationships influences the competitions between two viruses: 1) for attachment of infecting particles to the host cell at the time of inoculation and 2) subsequent multiplication of each after attachment. A third type of competition between viruses exists viz. the speed of spread to the growing points of the plant which includes also the relation of the two viruses to translocation facilities.

In the relation of the virus to the cell the first critical stage is the attachment of the infecting particles to the virus receptors (also designated as « sites ») of the cell (BURNET, 1953).

Attachment is the result of adsorption forces (POLLARD, 1953). The blocking of the receptors by one virus makes it impossible for another virus which requires the same receptors, to become attached unless its adsorption forces (affinity) are such to enable the latter in the competition to oust the former.

For a period after attachment the inoculated virus can not be detected (dark phase). It is supposed that the virus particles are disintegrating, forming sub-units which are assimilated by the enzymatic systems of the host cytoplasm. The hypothesis is advanced that for further multiplication the nucleic acids are the essential components (MARKHAM, 1953).

In the synthesis the idea of moulds or templets has been proposed (JANSSEN, 1939, KAVANAU, 1949). Long and short range specific attraction forces bring about the adsorption of the building blocks to these templets. The centers of increase, where normal enzymatic processes are changed, are thought to be the mitochondria (WOODS and DUBUY, 1943).

The virus arising can be considered as a competition of different particles of more or less infectivity accompanied by byproducts of synthesis MARKHAM and SMITH, 1946; TAKAHASHI and ISHII, 1953; BAWDEN, 1953).

This complex which is built up of a great number of different interrelated sub-units will come into a complicated relationship with the sub-units of the second virus (e.g. forming « hybrids » in special cases of bacteriophages) and with components of the host cell.

One can suppose that this relationship either results in a loss of infectivity of one of the viruses, or stimulates the increase of one virus to the other.

Supposing that viruses are in constant process of rebuilding and breaking down some sub-units may be interchangeable with metabolites of the cell and mutated viruses may arise.

Regarding virus translocation to the growing point the hypothesis is brought forward that sub-units are transported (ZECH, 1953). In most cases phloem is probably the important path of translocation; here conditions may prevail which are more favourable for the sub-units of a given virus than for those of another, e.g. with respect to pH regarding iso-electric point (OSTER, 1951). Another possibility is: transport of a virus may be the result of virus synthesis within phloem elements. In this case competition will also result in the above mentioned interferences.

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VIRUS STRAIN INTERFERENCE AND ITS RELATION TO SYMPTOM MANIFESTATIONS OF THE PSOROSIS DISEASE OF CITRUS

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Because a common characteristic symptom effect appears on young leaves of *Citrus* trees infected with the viruses which cause psorosis A, psorosis B, concave gum, blind pocket, crinkly leaf and infectious variegation, these diseases have been grouped under the name « *psorosis* ». On the basis of this and other similarities, it has been assumed that the different psorosis types are caused by related virus strains. Sweet orange trees inoculated experimentally with psorosis A, or grown from buds carrying psorosis A virus, display only the transitory young-leaf symptoms for a period of years but later the localized « scaly-bark » lesions begin to appear. If non-lesion tissue from such trees is used to inoculate healthy trees, the reaction is as just described. However, if the inoculations are made from bark taken directly from lesions the trees react quite differently. Young-leaf symptoms appears as usual, but additionally, a pimply, thin flaky type of bark lesion develops generally over the trees within three months and as the leaves mature they display rings, blotches, and brownish, raised spots. Considerable die-back of the twigs and limbs accompany this reaction.

Trees systemically invaded with the virus of psorosis A as a result of inoculation from non-lesion bark were reinoculated from lesion bark. These developed none of the early, general, severe symptoms which occur on healthy trees inoculated from lesion bark. This protection phenomenon provided an experimental test for relationship of the various psorosis strains. Test trees first infected from non-lesion bark of trees with psorosis A, psorosis B, and crinkly leaf, the lesion forming strains of psorosis, and from normal bark of trees with concave gum, blind pocket and infectious variegation, were reinoculated using psorosis A lesion bark. The second inoculation had no immediate effect on any of the previously infected trees. These results, repeated many times, demonstrate that the different types of psorosis are caused by related virus strains.

Another interesting reaction developed from these studies. With the knowledge that under natural conditions trees grown from buds infected with psorosis A are systemically invaded with the virus, it was puzzling as to why such trees regularly develop lesions spontaneously later in their life. In other words, it would seem that if the protection phenomenon existed there should be no naturally occurring lesion stage of this virus disease. Further study partially explained this apparent anomaly.

On trees which were protected when reinoculated from lesion bark, localized lesions began to develop in about two years. These lesions always originated at, or in close proximity to, the places where the lesion bark was inserted when the trees were reinoculated. As these lesions increased in size, they assumed all of the characteristics of the localized lesions which occur on infected trees under natural conditions which are not reinoculated. Thus, the experimentally infected trees, although protected against the early severe reaction, were not prevented from developing the typical lesions later. The two lesion types were sufficiently distinct to suggest that the *psorosis A* virus consisted of two components, one capable of inducing a localized lesion, — the other a general lesion. Results of studies to date indicate that the two lesion types are not caused by different virus strains or components. Instead, it seems that the early developing, general lesions are the reaction of a healthy, virus-free tree which permits rapid spread and multiplication of the virus inoculated into it. The same inoculum placed into a tree already invaded by virus from a non-lesion source is perhaps inhibited from rapid multiplication and spread through the plant. In such trees, however, it appears that the lesion inoculum can slowly invade the tissues at the point of inoculation, gradually overcoming the protective effects of virus already there and eventually reaching a concentration sufficient to cause injury.

Certain virus-host reactions indicate that the *psorosis A* virus may actually consist of a bark-lesion-forming component and a component which normally does not induce lesions. If this is true there are apparently other interference phenomena operating which as yet are not clearly understood.

THE ORIGIN AND INTERACTION OF FORMS OF THE PEACH MOSAIC VIRUS

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The peach mosaic virus appears to be continuously mutating to give rise to new forms distinguished by their differential expression on peach. Under conditions of orchard spread, individual trees of a single peach variety vary in symptom expression from severe dwarfing and rosetting to obscure flecks in leaves. Transmission from such affected trees result in kind to the symptoms from which the inoculum was taken, thus demonstrating that differences in expression are due to differences of the causal virus and not of the host.

When individual peach trees are infected with virus forms which produce severe symptoms, they develop characteristic severe symptoms during the initial stages of the disease. Late in the growing season of the first year of infection and commonly in the second and succeeding growing seasons, such trees develop sport-like shoots, usually from lateral buds, which are easily seen because of their vigorous growth. These show a range of symptoms milder than those in the remainder of the tree. Transmission from such individual shoots results in kind to the symptoms expressed on the shoot from which the inoculum was taken, demonstrating that the sport-like shoots represent the existence of different virus forms. It thus appears that the original virus form infecting the tree has given rise to new forms.

When trees infected with mild symptom producing forms are subsequently inoculated with tissue from the severely affected portion of the tree on which the mild form arose, no additional effect results. No additional effect should be expected because if the severe form had been able to invade the sport-like shoots on the mother tree the sport-like shoots would not have developed. In all cases transmission attempts from the re-inoculated trees resulted in only mild symptoms, indicating that the parent severe form had not produced infection in the re-inoculated trees.

When mild forms are matched with severe ones from widely separated geographic areas, a gradient in protection from complete to none occurs. In one case where Mild A protected against Severe A but not Severe B, and Mild B protected against Severe B but not Severe A, a combination of Mild A and B gave protection against both Severe A and B. In this case Mild A appears not to have prevented infection by Mild B in the same tree. In another case trees infected with a mild form characterized by small flecks and chlorotic spots on the lateral veins of the leaves were re-inoculated by a form characterized by production of severe mottled patterns and leaf distortion. The small flecks and chlorotic veins characteristics of the mild form were increased in size and intensity on the re-inoculated trees but there was no mottle and leaf distortion similar to that characteristic of the severe form alone in trees. In this case the mild form prevented normal development of the second form, but did not completely prevent infection by it. The protection afforded by mild forms appears to indicate relationships to the severe ones and the protection can be interpreted in terms of competition rather than to antagonism, the closer the relationship or likeness the more plausible they would use the same materials from the plant for reproduction.

If the mild forms arise by virus mutation from those producing severe symptoms, it seems reasonable that the reverse reaction should also occur. Forms producing severe symptoms would suppress growth and thus in a mildly affected tree would be obscured. When buds from severely affected

trees are placed on trees affected by related mild forms and forced into shoot growth, they produce typical severe symptoms but are soon overgrown by the mildly effected growth of the tree. When a series of trees are infected from a single stock culture of a mild form by inoculating each tree with a single shield bud, an occasional tree will develop more pronounced symptoms than the others. This suggests a reverse mutation from mild to severe, which may have been localized in the inoculum without expression. These cases have been too few to dissociate from possible natural reinfection by a form to which the mild one used did not afford protection, especially since the work was done in field plots.

The use of mild forms of the virus as protectants in peach trees seems promising as a control measure, especially in areas where peach mosaic is widespread in orchards and other means of control are ineffectual. Some mild forms have been obtained which appear to give good protection against all the forms producing severe symptoms with which they have been matched. If new forms arise against which the mild ones do not protect, they can be grown and mild mutant forms obtained from them.

COMMUNICATIONS DIVERSES

SOME ASPECTS OF THE SEROLOGICAL DIAGNOSIS
OF VIRUS DISEASES OF PLANTS

by E. VAN SLOGTEREN

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The great value of the serological methods for the study and the control of virus diseases of plants is apparent to all who have used them. The somewhat complicated technique however has been too much of a deterrent for general usage of serology in this field of biology.

The limitations of its application must not be a reason for the neglect of its possibilities. Every method of scientific research has its initial difficulties that have to be mastered.

Merits: 1. a quick and objective diagnosis, independent of the symptoms of the diseased plants; 2. the possibility of the identification and classification of many different viruses; 3. value of serology as an aid in the breeding of immune varieties; 4. use of serological methods in the study of the localisation of the virus and the virus-transport in the plants; 5. the possibility to determine the concentration of the virus in different stages of development of the plant; 6. finally the possible detection of yet un known but important virus diseases (e. g. the S-virus in the potato crop). The advantages of serology are far too great not to be accepted, even though often difficulties may arise in its application.

Limitations: 1. the failure to get an effective antiserum may be due to unstability of the virus in the expressed plant juice. The application of some reducing chemicals had been of some use, (LIMASSET, VAN SLOGTEREN Jr); 2. the presence of toxins in the plant juice may be fatal to the infected animals; the purification and the concentration of the virus without the loss of its antigenic activity sometimes has been possible; 3. the spontaneous flocculation of the plant juice has been most often the reason for a failure of serological reactions. In some cases these difficulties have been overcome by adding certain chemicals to the plant juice or by changing the pH of the solution. In other instances the virus has been successfully propagated on other host plants.

VAN SLOGTEREN Jr has applied the so called « geldiffusion » method, used by OUCHTERLONY and BJORKLUND, and to the study of the serological reactions of small-sized plant viruses. This method also may give some

idea of the size and the form of the virus particles and also of their mutual relation.

For the use of the serological reactions for the control of virus diseases and the selection of virus-free stocks in horticultural- and agricultural crops, an agglutination reaction has been worked out. Yearly in the Netherlands this method has been used for the testing of about a million motherplants. This is done for the testing on Virus X and Virus S of potatoes, and it has been possible to prepare a polyvalent antiserum for the testing of Viruses X, S, Y and Aucuba in one reaction.

The mass production of an antiserum to potato virus X has been made possible by the use of horses. The method used was an intravenous injection of virus that had been concentrated and purified from sap of infected tobacco plants. When injection of the antigen was done subcutaneously there was much less danger of the loss of the animal by shock, but the resulting antiserum contained only incomplete antibodies and was of no use for an agglutination reaction.

Production of an antiserum to potato virus S was accomplished by injecting rabbits with virus that had been concentrated and purified from sap of infected potato plants.

It has been possible to eliminate the toxic fraction of potato juice (toxic substances always being present in potatoes) by precipitating the virus with acetone of low temperature or using chloroform extractions of the sap.

About twenty different effective antisera have been prepared in the Laboratory for Flowerbulb Research at Lisse, among them an antiserum to the virus yellow disease of sugar beets, that has served to detect the presence of this virusdisease in the United States of America.

SOME EXPERIMENTS ON THE SEROLOGY OF POTATO LEAF ROLL VIRUS

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Very little information is known concerning the preparation of an antiserum against the potato leaf roll virus and to date all trials reported in the literature have yielded negative results. Generally speaking it is very difficult to obtain antisera against non-sap transmissible viruses. The reasons

why such a virus as leaf roll cannot be transmitted by sap are not known, but some possibilities might be as follows :

- 1°) The virus must be introduced into the phloem which is not possible by manual inoculation ;
- 2°) the virus may be very rapidly inactivated *in vitro* ;
- 3°) leaf roll virus occurs in very low concentrations in the plant ;
- 4°) a combination of the above three possibilities may be operating.

In preparing an antiserum point 1 needs not be considered. If it is a question of virus inactivation in the sap (point 2) there is little likelihood of success in preparing an antiserum. On the other hand if low concentration of virus (point 3) is the principal factor involved, there might be some possibility of producing a low titre antiserum.

Following is described a method by which positive results were obtained in preparing such an antiserum. One of the most useful experimental host plants of the leaf roll virus is *Physalis floridana* because it is so easily infected and apparently produces a higher concentration of virus. Crude sap from infected plants of *P. floridana*, which was dialysed for two to three hours in running tap water was injected intravenously into rabbits. Eight injections were made and one week following the last injection the serum was obtained in the ordinary way. The reactions were carried out in tubes in each of which was mixed 1/2 ml. serum and 1/2ml. clear centrifuged sap. During the tests temperature in the tubes was maintained at approximately 37°C.

The results may be summarized as follows :

1) It was shown that absorbing one part serum with one part healthy sap was sufficient to eliminate all the antibodies against normal plant proteins from the serum.

2) This absorbed antiserum, however, did not give any positive reaction when mixed (at any combination of dilutions) with clear centrifuged sap from diseased plants.

3) When unabsorbed serum, at dilutions 1 to 2 and 1 to 4 with saline (0.9 % NaCl) was reacted with sap, healthy and diseased, that had been diluted with water before centrifuging, no differences between healthy and diseased sap could be detected.

4) When, however, healthy and diseased saps were diluted with saline before centrifuging positive reactions were obtained with the diseased sap at higher dilutions than with healthy sap. The ultimate dilution of the serum at which there were positive results was 1 to 16.

Repeated trials have always given the same results, namely, the diseased sap always gives a reaction at a higher dilution than healthy sap.

In the following table are presented the experimental results :

		Diluted with water before centrifuging.						Diluted with saline before centrifuging.						
		Healthy			Diseased			Healthy			Diseased			Saline
		1/2	1/4	1/8	1/2	1/4	1/8	1/2	1/4	1/8	1/2	1/4	1/8	-
Antiserum diluted	1/2	-	-	-	-	-	-	+	-	-	+	+	+	-
	1/4	-	-	-	-	-	-	-	-	-	+	+	+	-
	1/8	-	-	-	-	-	-	-	-	-	+	+	+	-
Antiserum absorbed	1/2	-	-	-	-	-	-	-	-	-	-	-	-	-
	1/4	-	-	-	-	-	-	-	-	-	-	-	-	-
Normal- serum diluted	1/4	-	-	-	-	-	-	-	-	-	-	-	-	-
	1/8	-	-	-	-	-	-	-	-	-	-	-	-	-
Saline		-	-	-	-	-	-	-	-	-	-	-	-	-

From the above it can be seen that the possibility exists to diagnose the leaf roll virus in *Physalis floridana* by serological means. Whether the positive reactions with diseased sap are due to a reaction with the leaf roll virus or an excess of normal proteins in diseased sap can not be stated at present.

If an antiserum has been produced against the leaf roll virus it must be concluded that the virus is present in the sap in very low concentration (diseased sap diluted 1 to 16 does not give a positive reaction) or has little antigenic activity. It appears that when diseased sap is diluted with water the virus must be precipitated whereas on the other hand saline tends to keep at least part of the virus in the supernatant during centrifugation. Reactions with serum that had been saturated with healthy sap were negative with both diseased and healthy sap. Possibly a portion of the antibodies to the virus, which are present in very low concentration in the serum, are precipitated along with the healthy antibodies. Thus the residual concentration is too low to give a positive reaction.

PROBLEM OF DEGENERATION OF POTATOES AND EPIDEMIC PHENOMENON OF VIRUS DISEASES

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Two elements constitute the virus disease in plants, namely infection which is brought about by foreign nucleoprotein molecules and secondly

the individual reaction of the cell substances which manifest themselves exteriorly by different symptoms of the disease. This lecture treats in the first instance of the correlation which exists between climate and the spread intensity and external symptoms of two very frequent virus diseases in potatoes, namely the virus X and virus Y.

The potatoes were observed in the experimental fields of Cracow and Zakopane, near Tatra Mountains (about 900 m.) from 1947 — 1953. In the lowlands as well as in the mountains the climate was subject to prominent fluctuations in the periods of the growth of the plants during these 7 years. In Cracow the year 48, 49 and 52 were comparatively cool and humid, whereas the years 47, 50 and 51 were distinctly dry and hot. In general in the heart of summer the climate of Cracow can be characterised as a « steppe » climate. The amount of daily evaporation is higher than the sum of rainfall. At Zakopane in contrast with Cracow these circumstances are just the reverse, the rainfall surpasses manifold the evaporation. At Zakopane only the year 1950 was comparatively dry.

Since 1947 tubers of potatoes have been imported every year from experimental stations of Pomerania. There these potatoes had not shown any symptoms of virus diseases and their development was optimal. Thanks to vegetative propagation the development and health of every plant was studied in Cracow and at Zakopane during all the following years. The plants showed symptoms of virus diseases as well as general degeneration without specific symptoms of virus diseases.

In all the successive generations of potatoes brought in from Pomerania the percentage of degenerated plants attained the highest level (60 %) in hot and dry years.

In Cracow the potatoes were continually colonised by aphids, in consequence of which they were permanently in danger of being infected. In spite of that the generations of potatoes imported in different years planted in mutual neighbourhood underwent the infestation of virus Y in different ways. The virus Y infested in the year 1949 epidemically only these potatoes which grew in Cracow in the dry year 1947. The potatoes imported in the years 1948, 1949 were not infected by the virus Y. This disease reached its height in these potatoes in the year 1952 and 1953 after the drought which reigned in the years 1950 and 1951.

The potatoes infected in Cracow in 1947 by the virus Y and transplanted to Zakopane near Tatra Mountains, have only manifested no epidemic in 1949 but they have masked its symptoms already that year and many plants got rid of it completely in the following year.

From these observations we may conclude that :

1°) The biological state of the potatoes plant is a more prominent element associated with the infestation by virus Y than the infection factor itself.

2°) The epidemic intensity was in our experiments a consequence of the degeneration of the potatoes plants in the previous year owed to the conditions of the climate.

3°) Mountain climate masks the symptoms of virus Y and diminishes partly the infestation itself.

Biological and serological observations of potatoes virus X of the same potatoes have given the following results.

1°) The increase of virus X in the potato tissu undergoes alterations in correlation with the changes of the vital conditions of the plant. Potatoes transplanted from Pomerania to Cracow undergo an increase of virus X.

2°) The strains of virus X interchange mutually in correlation of the qualities of the given variety of the potatoes and of the climatic conditions. Mildly virulent strains pass into strongly virulent ones and vice versa.

3°) Mountain climate in correlation probably with insolation does not allow the strongly virulent strains of virus X to develop.

The development of the infectious disease in plants which degenerated in the previous year owing to dry climatic conditions is connected with the problem concerning the transmission of the plant biological potential through vegetative propagation. The potatoes which since 1948 were cultivated in the Tatra Mountains (about 960 m.) were transplanted in the years 1950, 1951 and 1952 to lowland in the neighbourhood of Cracow (200 m.). A comparison was made of the yields given by three generations of potatoes, namely: 1° potatoes cultivated in Cracow since 1947, 2° plants transplanted for the first time in 1948 to Zakopane, and cultivated there until 1952, 3° plants that were transplanted back from the mountains to the low - land. The difference in the yields shown in the three above cases were very marked. The mean amount and weight of tubers transplanted from the mountains to the low - land were three and four times greater than the amounts and weights of tubers originating from plants cultivated either in the mountains or in the low - land without interruption. Potatoes planted in the Tatra Mountains were spared the period of dry climatic conditions. This circumstance exerted a beneficial influence on the development in the following year, despite the unfavorable growth conditions.

ELEKTRONENOPTISCHE UNTERSUCHUNGEN UEBER DAS BLATTROLLVIRUS DER KARTOFFEL

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Im Jahre 1951 nahm ich die elektronenoptische Untersuchung des Blattrollvirus auf. Als Ausgangspflanzen dienten 6 rollkranke und 5 ge-

sunde Augenstecklinge, die mittels *Physalis floridana* auf das Blattrollvirus getestet worden waren. Die Freiheit von verschiedenen anderen Viren wurde mittels einer Reihe weiterer Testpflanzen nachgewiesen. Die oberirdischen Teile der Pflanze wurden gepreßt und die Pressäfte nach verschiedenen Methoden zu reinigen versucht. Nachdem der Versuch, einen Teil des Saftes mit $(\text{NH}_4)_2\text{SO}_4$ zu reinigen, fehlschlug, wurde der Rest des Saftes jeder Pflanze in 3 Teile geteilt und jeder Teil verschieden behandelt.

Der *erste* Teil wurde sechsmal bei 3500 Umdrehungen und je 40 Minuten Laufzeit zentrifugiert. Die Präparate für das Elektronenmikroskop wurden durch Eintrocknenlassen des underdünnten überstehenden Saftes auf einer Kollodiummembran hergestellt und mit Palladium schräg bedampft. Bei der elektronenoptischen Untersuchung zeigten sich auffallend lange, dünne Stäbchen. Sie zeichneten sich durch eine etwas ungleichmässige Dicke und vor allem durch eine charakteristische Bündelung aus. Interessant ist weiter das Vorkommen kleiner « Stäbchennester ». Die Länge der Stäbchen liegt zwischen 150-4250 $\text{m}\mu$. Die stäbchenförmigen Gebilde konnten nur in dem Preßsaft einer einzigen rollkranken Pflanze gefunden werden. Allerdings waren die übrigen Säfte derart stark verunreinigt, dass eine genaue elektronenoptische Untersuchung nicht möglich war.

Der *zweite* Teil der Preßsäfte wurde zunächst einmal bei 3500 Umdrehungen und 40 Minuten Zeitdauer zentrifugiert und anschliessend in einer Ultrazentrifuge bei 50.000 Umdrehungen 40 Minuten lang behandelt. Der überstehende Saft zeigte keine typischen Gebilde im Elektronenmikroskop. Der Niederschlag, der in dest. Wasser aufgenommen wurde, ergab selbst bei grösserer Verdünnung nur stark verunreinigte Bilder.

Im *dritten* Teil des Preßsaftes, der in gleicher Weise wie der zweite behandelt worden war, wurden Niederschlag und überstehende Flüssigkeit des Ultrazentrifugates wieder intensiv gemischt und geschüttelt und das ganze bei Zimmertemperatur acht Tage lang stehen gelassen. Dadurch trat eine Ausflockung ein, die durch 6 maliges Zentrifugieren bei 3500 Umdrehungen und 40 Minuten Laufzeit ganz zu Boden geschlagen wurde. Die überstehende Flüssigkeit wurde zur Herstellung eines Präparates verwandt, das in der oben beschriebenen Weise angefertigt wurde. Die Untersuchung im Elektronenmikroskop ergab das Auftreten von gleichmässig dicken, stäbchenförmigen, oft gewinkelten Gebilden, deren Längen, wenn man die Summe der Kniskstücke als Gesamtlänge wertet, zwischen 66 und 2300 $\text{m}\mu$ schwanken. Die verschiedenen Längen sind keineswegs immer ganze Vielfache der kleinsten Einheit. Die Stäbchen wurden in den Preßsäften von 4 der 6 rollkranken Pflanzen gefunden, während in dem Preßsaft der gesunden Pflanzen diese Gebilde nicht zu beobachten waren.

Ob die stäbchenförmigen Gebilde, die im ersten Teil des Preßsaftes beobachtet wurden, gleicher Herkunft sind, wie die im dritten Teil des

Preßsaftes festgestellt, ist nicht leicht zu entscheiden. Dass die Bündelung und Nesterbildung im dritten Teil des Preßsaftes nicht mehr in der charakteristischen Form auftraten, lässt sich vielleicht mit der verschiedenen Behandlung der Säfte erklären. Die verschiedenen Stäbchenlängen würden jedoch nicht gegen eine Uebereinstimmung sprechen. Es lässt sich denken, dass die im Preßsaft beobachteten langen Stäbchenformen durch das Zusammentreten kleinerer Einheiten entstanden sind. Diese Aggregatformen aber könnten wiederum mit dem Austreten des virushaltigen Preßsaftes aus den Zellen bzw. dem Phloem und dem gleichzeitigen Zusammentreffen mit der Luft zusammenhängen. Wäre nun die Infektion beim Blattrollvirus, wie dies z. B. für das Tabakmosaik wahrscheinlich gemacht wurde, an eine bestimmte Teilchengröße gebunden, so ließe sich dadurch die Lichtübertragbarkeit des Virus durch Preßsaft und die Infektionsmöglichkeit durch Läuse und Pfropfung erklären.

Wie sich bei späteren Untersuchungen gezeigt hat, ist die Auffindung dieser Gebilde in blattrollkranken Pflanzen, selbst bei genauer Einhaltung der ursprünglichen Präparationsmethode, durchaus nicht immer möglich. Trotzdem spricht aber das ausschließliche Vorkommen dieser Gebilde bei rollkranken Pflanzen dafür, daß diese Formen in einem Zusammenhang mit dem Blattrollvirus stehen. Erhärtet wird diese Annahme dadurch, dass inzwischen der Nachweis der Stäbchen auch im Phloemsaft gelang sowie durch die spätere Beobachtung der gleichen Formen im Saft blattrollkranker Kartoffelnollen durch DR. KANNGIEßER (mündliche Mitteilung). Ob sie allerdings das Blattrollvirus selbst darstellen oder nur Formen, die unter seinem Einfluß in der Pflanze gebildet und durch die Präparationstechnik ev. verändert wurden, kann nicht entschieden werden, da die Rückimpfung durch den Saft blattrollkranker Pflanzen, der solche Stäbchen enthielt, nicht möglich war.

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Summary. — ELECTRON-OPTICAL RESEARCH ABOUT POTATO LEAF ROLL VIRUS.

For the research leafroll-sick and healthy potato plants have been used. The press-sap, which was gained from the overground parts of the plants, was treated with the ultra-centrifuge; then, leaving it in room temperature, a precipitate occurred and in a after-treatment it was purified with a normal centrifuge. Hence the pure sap, gained in the prescribed way, was undiluted electronoptical analysed by shadowing with palladium. In the saps of 4 of the leafroll-sick plants rod-like, straight or angular structures could be seen, varying in its length between 66 and 2300 m μ . In another sample (preparation) the press-sap was treated six times only with the normal centrifuge and the sticks, which were accumulated in bundles or in beds, showed a length between 150 and 4250 m μ . There is also a great probability that these rods are in coherency with the leaf-roll virus, because the same structures (particles) could not yet be found in healthy plants and similar structures were observed by me in the phloem sap and later by Dr. KANNIEßER also in the tubers of leafroll-sick plants. It is not yet decided if these sticks are identical with the leafroll virus or if they are only structures, formed in the plant by influence of the virus.

THE MUTATIONS OF THE POTATO VIRUS X INDUCED BY ULTRASONICS

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Problems concerning the mutability of the Potato Virus X are of a great practical and theoretical value. We know at present many strains of this virus which differ greatly in the severity and patterns of symptoms, they cause on the infected test-plants. Many facts are already stated which suggest, that these strains can mutate one into another, when influenced with some physico-chemical agent ex. light, temperature. Nevertheless the mechanism of action of these factors is till now quite unknown, it is clear, that the possibility of inducing here strictly definite changes in a controlled direction, may contribute much to the understanding of whole this problem.

In the following work I investigated the possibilities of inducing mutations of the Potato Virus X by ultrasonic treatment. Extracts of tobacco-plants juice, infected previously by a necrotic strain of virus X, were sonised by ultrasonic apparatuses of various acoustic power, during different lapses of time. The results of ultraacoustic treatment were investigated

by the inoculation of the sonised juices on test plants. Some changes in virulence of the sonised tobacco extracts, were stated. Extracts which previously produced severe necroses, when inoculated on the tobacco leaves, evoked often, after sonic treatment, only mosaic or mottle symptoms. In many cases no external symptoms were visible at all. The changes in external symptoms, obtained after sonisation, remained in most cases stable, when reinoculated on fresh test plants. The reduction in virulence of the sonised extracts proceeded proportionally with the time of the acoustic treatment. It was strictly correlated with the type of the ultrasonic generator which was used. Extracts sonised by applying acoustical power about 5 Watt/cm^2 , produced strikingly less severe symptoms on the tobacco leaves, than those, treated by a generator emitting only about $1,2 \text{ Watt/cm}^2$ of acoustic energy. The majority of plants, which after inoculation with the sonic treated extracts showed no visible symptoms of virus infection, gave positive results in complement fixation or precipitin tests. Virus extracts partly purified of healthy proteins were much more sensitive to the acoustical treatment, than the raw ones.

They reacted also more uniformly.

Two problems must be solved: 1) To what degree the observed changes of symptoms can be ascribed to real mutations in the potato virus X strains? 2) If so be, remains to know, if the influence of sonisation has only the character of selectionning the given types of virus strains, or whether it intrinsically induces mutations of one strain into another. Results of this investigation can only throw some light on both these problems. As to the first problem one cannot exclude the possibility of having dealt with real mutations of the virus X. The methodical conditions of experiments however, as well as their field, do not allow to solve this problem definitely. In regard to the second question we can only state that some of the types of symptoms, evoked on tobacco by virus X infection, were specially sensitive to the kind of the ultrasonic wave. So the application of weak acoustical powers favoured strongly the milder types of virus X symptoms, whereas extracts treated with 5 Watt/cm^2 of acoustic power produced, on infected tobacco plants, no more symptoms of disease in most cases. It is evident, that even a pretty strong sonisation does not denature completely virus protein, which is still partly capable of reproduction. It produces however no more symptoms of diseases on the infected plant.

THE USE OF RADIOISOTOPES IN STUDYING THE INTERACTION BETWEEN FUNGUS SPORES AND TOXIC CHEMICALS

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In studying the mechanism of the action of fungicidal chemicals it is important to know the actual amount required to be taken up by fungus conidia to bring about their destruction. It is also interest to learn whether the fungicidal chemicals undergo changes when taken up by spores and whether host plants take up and metabolize fungicides. Investigations of this nature have been made much easier with the availability of radioisotopes since very small amounts of chemical can be determined quantitatively. In metabolism studies the tagged element can be followed even after it has undergone chemical changes.

Experiments along these lines have been conducted at the Boyce Thompson Institute in cooperation with the United States Atomic Energy Commission for the past several years. Isotopes of sulfur, silver, mercury, and cerium, and C^{14} -labeled 2-heptadecyl-2-imidazoline and ferric dimethyldithiocarbamate labeled with S^{35} have been used. Conidia of 12 species of fungi have been included in the studies.

In tests in which conidia were exposed to dilute solutions of various toxicants in 15-ml. tubes, interaction between the spores and the toxicants was found to be marked and rapid. With sulfur the results differed from those of other toxicants in that there was a rapid reduction of the sulfur to hydrogen sulfide. There was no particular accumulation of sulfur in the spores; spores of species which did not have a high capacity for reducing sulfur to hydrogen sulfide did not remove much sulfur from the ambient solution. The results have indicated that hydrogen sulfide is comparatively non-toxic to spores. Spores of some species can produce large quantities of hydrogen sulfide without germination being completely inhibited.

Other toxicants studied were taken up by spores rapidly from dilute solutions and such large accumulations were necessary to affect marked reduction in germination. With 1-heptadecyl-2-imidazoline accumulation could amount to 10,000-fold or more. Over a wide range the amount of toxicant taken up by spores treated with the imidazoline was determined by the amount of toxicant present in relation to the quantity of spores. Toxicant taken up, with silver and cerium as well as with the imidazoline, were largely completed in the first 30 seconds or less for exposure. ED50 values

obtained ranged from 85 p.p.m. of spore weight for silver and spores of *Venturia pyrina* to 9300 p.p.m. for the for the imidazoline and spores of the same species.

In extensive experiments in which the effect of 2-heptadecyl-2-imidazoline, silver, and cerium was studied in simultaneous and consecutive treatments, the results indicated that the receptor sites for these three toxicants were different. There was no interference, as far as uptake or subsequent release was concerned. When materials more closely related chemically were tested there naturally was an indication of similar or identical receptor sites. The rare earth elements, neodymium, lanthanum, samarium, and praseodymium, were found to act similarly to cerium. The receptor sites for all these rare earth elements seem to be identical.

In experiments in which the effect of Hg^+ , Hg^{++} , Cu^{++} , Cd^{++} , Ni^{++} , Co^{++} , and Zn^{++} ions on the uptake and release of Ag^+ by spores of *Neurospora sitophila* was studied, Hg^+ and Hg^{++} ions had the greatest influence. In these tests, pre-treatments, simultaneous treatments, and the release on subsequent treatments were investigated. The various ions exerted their greatest effect when simultaneous treatments were made. The addition of large amounts of non-radioactive silver showed that silver taken up by the spores readily exchanges when more silver is added. Exchange does not seem to occur so readily with cerium or 2-heptadecyl-2-imidazoline.

The experiments with mixtures of toxicants may have some practical significance. If the toxicants used are not closely related chemically there is no interference of one toxicant with the other. If they are related such interference does occur. There is some evidence that spores treated with one toxicant to a degree which would result in inhibition of germination, may be benefited by treatment with a less toxic material which partially displaces the first toxicant.

MULTIPLICATION DU VIRUS DE LA MOSAÏQUE DU TABAC EN CULTURE DE TISSU

par L. HIRTH et G. SEGRETAÏN

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Le virus de la mosaïque du tabac se multiplie en culture de tissu de crown-gall de tabac entretenue depuis plus de trois ans sur milieu minéral sucré gélosé. Différentes substances (lait de coco, acides aminés, bases puriques et pyrimidiques) sont incorporées au milieu et leur action sur la multiplication du virus est étudiée.

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ACTION DE L'ACRIFLAVINE SUR LE VIRUS
DE LA MOSAÏQUE DU TABAC *IN VITRO* ET *IN VIVO*

par L. HIRTH

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L'action de diverses concentrations d'acriflavine sur le virus de la mosaïque du tabac est étudiée *in vitro* et la virulence de ce dernier est évaluée au moyen des lésions locales obtenues sur *Nicotiana glutinosa*. L'influence de l'acriflavine sur la multiplication du virus de la mosaïque du tabac en culture de tissus de tabac et dans des fragments de feuilles isolées est éprouvée; le virus est dosé au spectrophotomètre de Beckmann. L'action conjuguée de l'acide tannique et de l'acriflavine sur le virus de la mosaïque du tabac est également étudiée.

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LE NANISME DES PLANTES VIROSEES :
ANALYSE PHYSIOLOGIQUE
DES MECANISMES D'ACTION DES VIRUS

par J. PAVILLARD

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1. — Par des extractions à l'éther, de durée brève, à 0° C., éliminant tous risques de destruction de l'auxine par les diastases oxydantes qui sont extrêmement abondantes chez les plantes virosées, j'ai *confirmé* en tous points les résultats antérieurs de Söding et ses collaborateurs concernant la teneur en auxine des Pommes de terre virosées. Quelle que soit la virose : mosaïque à virus X, enroulement, frisolées complexes, la présence des virus est toujours accompagnée d'une *réduction importante* de la teneur en auxine des tissus malades, que j'ai appelée l'*hypoauxinie* (Pavillard 1953).

2. — Par une technique spéciale d'hydrolyse alcaline libérant l'auxine « liée », j'ai montré que, dans les tubercules virosés, la quantité de « *précurseur d'auxine* » est très inférieure à celle des tubercules sains. Ce fait, joint à l'hypoauxinie notée précédemment, permet d'expliquer facilement la poussée moins vigoureuse et la loi de développement différente des

Pommes de terre atteintes d'Enroulement, phénomène bien analysés par Bald et Hutton (1950).

3. — J'ai montré que les mêmes phénomènes se retrouvent exactement chez la Tomate atteinte de Mosaïque du Tabac ou de virus X ou d'un mélange des deux virus. Tout ceci, joint aux faits signalés par Griève (1943 a) pour la Tomate atteinte de la Maladie bronzée, permet de conclure que l'*Hypauxinie est une propriété physiologique générale caractérisant les maladies à virus*, ce qui les OPPOSE aux *maladies hypertrophiantes, cryptogamiques ou bactériennes*, au cours desquelles les organes déformés et les territoires voisins deviennent très fortement HYPERAUXINIQUES. (Link et coll. 1937, Moulton 1942, Griève 1943 b, Pilet 1952, 1953), et les RAPPROCHE étroitement des *carences* où l'hypauxinie a été bien décrite. (Skoog, 1940, Cheng-Tsui 1948, Avery et coll. 1936, 1937; Avery et Pottorf 1945).

4. — J'ai montré ensuite, sur des Tomates inoculées mécaniquement par le virus de la Mosaïque du Tabac, que l'*hypauxinie est la cause directe* du ralentissement de la croissance (conduisant au nanisme des plantes virosées). J'ai, pour cela, étudié, parallèlement à l'apparition du nanisme, la genèse de l'hypauxinie. Ces deux phénomènes varient constamment en parallèle, et débutent *avant* que les premières taches de la panachure mosaïque n'apparaissent sur les feuilles. Plus précoces que l'atteinte faite aux capacités photosynthétiques des feuilles, ils sont donc *indépendants* d'une réduction possible de l'intensité assimilatrice.

5. — L'étude simultanée de l'expansion du virus de la Mosaïque du Tabac à partir de son point d'inoculation, et des variations de la teneur en auxine des diverses feuilles chez les Tomates inoculées a permis de *souligner* l'étroite liaison qui existe entre l'*apparition du virus* dans une feuille quelconque (seuil de floculation de l'immunsérum spécifique par la *Micro-méthode sérologique* de Limasset et Augier de Montgremier 1947, 1948) et la manifestation d'un *état d'hypauxinie* de cette feuille. Il faut que le virus soit présent pour que se manifeste une telle hypauxinie.

Or, les recherches de Wildman, Cheo et Bonner (1949) ont établi que, chez le Tabac, le virus de la Mosaïque du Tabac se forme aux dépens du *composant protéique principal du cytoplasme foliaire (main protein component)*. Puisque ce composant est le *support de l'auxine* (Wildman et Bonner, 1947) il est naturel que la substitution de la protéine du virus à une fraction importante de ce composant se traduise par une diminution, du même ordre de grandeur, de la teneur en auxine des feuilles, ce qui a pour effet de provoquer un ralentissement de la croissance conduisant au nanisme des plantes virosées.

Ce *nanisme* est donc un caractère SPÉCIFIQUE de l'action des virus, et non pas, comme on l'admettait jusqu'ici sans l'avoir démontré, une conséquence secondaire des troubles nutritifs engendrés par la présence du virus.

6. —L'étude comparée des courbes de croissance de tomates mosaïquées poussant à des époques différentes de l'année, suggère qu'à côté de cette action directe du Virus de la Mosaïque du Tabac sur le *complexe auxinique actif*, il doit exister, dans les périodes peu favorables à la croissance, une autre action perturbatrice du virus, liée à l'existence de facteurs SPÉCIFIQUES de la croissance des limbes foliaires DISTINCTS de l'*auxine* (*phyllocaline* de Went, 1938, 1951). Au *nanisme* « PRIMAIRE » décrit ci-dessus, et d'origine strictement hypoauxinique, se surimpose en effet un *nanisme* « SECONDAIRE » lié à une *forte réduction des surfaces foliaires développées*, après la fin de l'incubation.

CUCUMBER MOSAIC VIRUS STRAINS AND SOME OF THEIR PROPERTIES

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An investigation of four strains of the cucumber mosaic virus (*Cucumis* virus 1) has been conducted along premunity and serological lines, viz.

1. ordinary cucumber or gherkin mosaic;
2. a strain of the virus from *Chrysanthemum*;
3. a yellow strain (nr. 6 of Price).
4. a strain from *Primula veris* herebefore undescribed in the Netherlands.

The symptoms of the *Primula* virus on testplants and some of the properties of these viruses are discussed.

In many gardens in the Netherlands *Primula veris* is found as a perennial. An investigation that was started by THUNG and carried on by me showed that not only plants with flower-colour changes, but also many healthy appearing plants were infected with virus. On several occasions a virus was isolated which showed relationship to the cucumber mosaic virus. A week after sap inoculation of *Nicotiana tabacum* « White Burley » a faint mosaic mottling can be observed, which then disappears almost entirely a month after inoculation. These symptoms, as well as the symptoms on *N. glutinosa* and *Cucumis sativus* are very similar to symptoms of the gherkin mosaic virus. In *Primula wanda* the virus became systemic, but only a faint veinclearing developed.

Cross protection tests between the *Primula* virus and the yellow strain of *Cucumis* virus 1 demonstrated an interrelationship. Eleven of 12 tobacco plants and all 5 *N. glutinosa* inoculated first with the former virus failed subsequently to become infected with the latter. All 15 healthy plants

simultaneously inoculated with the *Cucumis* virus 1 became infected. In other experiments the yellow strain succeeded in infecting plants, which were infected first with the *Primula* virus. A possible explanation for these conflicting results is that protection against the yellow strain by the *Primula* virus is incomplete when a time of longer than 2 to 3 weeks (at temperatures of 20-25°C) elapses before inoculation with the yellow strain. This explanation proved to be valid in the experiment where 11 out of 12 plants were protected against the yellow strain. When inoculation with the yellow strain was made 3 weeks after the first all the 12 plants showed mixed symptoms of the two viruses. On the contrary none of the 5 *N. glutinosa* plants developed yellow symptoms after the second inoculation with yellow cucumber mosaic virus. One can not conclude on the basis of this that the two viruses are only distantly related since in one experiment even re-inoculation of plants, infected with the yellow strain for some weeks, with the same yellow strain also induced stronger symptoms than these on the once inoculated plants.

Many efforts were made to partially purify cucumber mosaic virus and to obtain positive precipitin reactions with the virus and its antiserum. Although this virus may remain active in sap for several days, on occasions an inactivation within a few hours has been observed, Köhler (1935), Nienow (1948), Noordam (1952). Another possible reason for the difficulties encountered in purification and serology is the fact that the virus is frequently sedimented by low speed centrifugation, Sill, Burger, Stahmann and Walker (1952).

Rapid inactivation of the yellow strain of cucumber mosaic virus was clearly demonstrated in an experiment. This virus which in tobacco sap had a dilution end point of 1 : 1000 was completely inactivated in one hour at 12°C and 3 hours at 0°C. This inactivation could be prevented by grinding the leaves with $\text{Na}_2\text{SO}_3 \cdot 7 \text{H}_2\text{O}$, 1 g to 100 g of leaves. In this way virus remained active and could withstand centrifugation at 9000 r.p.m. for 45 minutes without any loss of activity. Adding KCN (at an end dilution of 0,3 %) was not as effective as Na_2SO_3 .

In the serological work on the cucumber mosaic virus strain from *Chrysanthemum* it became evident that it is important how the sap is treated before centrifugation. Sap from diseased tobacco diluted with water before centrifugation repeatedly failed to give a serological precipitin reaction, whereas the same sap diluted with saline before centrifugation yielded a very distinct positive reaction. Although Na_2SO_3 is more effective in checking the inactivation of the virus than saline, only sometimes did we succeed in obtaining a positive serological reaction with sap centrifuged with Na_2SO_3 . Perhaps in these cases the virus concentration in the sap was too low, but this was not shown to be the case.

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TRENDS IN STONE FRUIT VIRUS RESEARCH

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Virus diseases of stone fruits are becoming as numerous as those in any of the other groups of commercial crops. Unfortunately, knowledge of their identities and relationships remains very meagre.

The stone fruit virus investigator has been unable thus far to adapt to his research most of the standard aids used in virus studies. Extraction and purification of causal viruses, and serological studies of them, have not been possible. Few of the diseases have known vectors. Cross protection is a rare phenomenon. However, a more serious handicap than any of these has been the difficulty experienced in obtaining pure cultures of the causal viruses, even in living hosts.

MILBRATH has suggested that over 95 % of the sweet cherry trees growing in Oregon carry latent ring spot viruses. Experience of workers in neighbouring districts such as British Columbia has indicated widespread occurrence of these ring spot viruses, and the almost equally ubiquitous occurrence of several other latent viruses. Each new indexing host employed reveals the presence of additional latent viruses, so that conclusive evidence for purity of cultures is difficult to obtain.

The common occurrence of mixed cultures not only prevents investigation of the component viruses, but also renders difficult the assessment of the significance of variations in the symptoms and behaviour of virus diseases from district to district, and even from tree to tree. These variations are a common phenomenon. Several good examples can be selected from the problems confronting our laboratory.

Lambert mottle and twisted leaf of sweet cherry are two of the most serious stone fruit virus diseases occurring in British Columbia. Each disease manifests significant differences in symptom expression from tree to tree in affected orchards. For each disease various source trees have yielded differing contents of latent viruses that, by present techniques, are inseparable. Determination of the causes of the symptom variation is, therefore, impossible.

In most of the fruit-growing districts on the Pacific slopes of North America there occur virus diseases that interfere with the normal ripening of sweet cherry fruits. At present five diseases are recognized; cherry buckskin, little cherry, albino cherry, small bitter cherry, and western X little cherry. The distinctions are based on regional distribution, together with small, but apparently significant differences in symptoms, in incubation periods, and rates of spread in infected trees, and in host ranges. When our laboratory has attempted to elucidate the relationships of the little cherry diseases with the diseases in other districts, at least three latent viruses have been encountered in little cherry source trees tested. It is difficult, therefore, to determine whether the differences that exist between any two of these small cherry virus diseases result from strain differences in the causal virus, or from the influence of differing combinations of latent viruses, forming complexes with a single causal virus.

Thus the assessment of the identities and relationships of stone fruit virus diseases, and the opportunity to study the viruses that cause them, are dependent on the development of techniques for the separation of viruses, and thereby the purification of cultures. A stage has been reached in stone fruit virus research that justifies the intensification of efforts to develop and employ such techniques, utilizing the closely coordinated contributions of plant pathologists, entomologists and chemists grouped in research teams.

Numerous techniques are available for development or elaboration. Profiting from recent demonstrations that certain viruses can be transferred from stone fruits to herbaceous plants, increasingly wide host ranges of herbaceous plants, as well as stone fruits, can be employed in a search for screening hosts. Taking encouragement from recent progress in the discovery of insect vectors, this search can be intensified and the demonstrated vectors manipulated by methods similar to those employed for separation of small fruit viruses. Recent demonstrations of the transmission of stone fruit viruses by means of dodder, of extruded juice, and of seeds, suggest that these may prove to be selective transmission agents. Rates of spread of viruses through host tissues can be studied, and differing rates exploited. Specific plant parts such as floral organs and roots can be selected as sources of inoculum. Heat treatments can be applied to host plants and vectors. Recent improvements in virus extraction methods suggest that more ingenuity can be applied to the extraction of stone fruit viruses with some promise of success.

Virus separation techniques have been receiving increased attention at most stone fruit virus research centres, including our laboratory at Summerland. They must be explored with greatly increased determination, because the extent to which they are successful should determine whether stone fruit virus research will be able to avoid an impasse and progress toward a position in which causal viruses can be identified and studied.

THE TRANSMISSION OF « INFECTIOUS CHLOROSIS »
OF MALVACEAE BY GRAFTING AND INSECT-VECTOR

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In our grafting experiments we tried to compare the behavior of our different sources or the virus of « Infectious Chlorosis » in several indicator species. In these experiments it was interesting to verify, that *Abutilon striatum* Dicks var. *Thompsonii* Veitch., which is characterized by a striking ornamental leaf-pattern, induces much milder symptoms in *Sida rhombifolia* L. than the native source of virus which is common in Brazil in weeds of this species. In spite of such slight differences in symptom-expression, our various sources of the virus of « Infectious Chlorosis » showed much agreement in the grafting experiments.

Making use of the insect vector *Bemisia Tabaci* we succeeded easily in transmitting the virus originally harboured in *Sida rhombifolia* (from São Paulo and Trinidad) to healthy plants. We could transmit the virus by one single white fly, caged over a leaf or even a flower of an infected *Sida* plant. In the course of these studies we verified, that the virus is persistent in the insect-vector, but that the manner of transmission is intermittent.

With the insect transmission of the virus of *Abutilon Thompsonii*, even if introduced by grafting to *Sida rhombifolia*, we experienced much greater difficulties.

TABLE ALPHABETIQUE DES AUTEURS

- | | |
|---|-------------------------------|
| KORF (R. P.), 80. | ARK (P. A.), 186. |
| KUCZYNSKA (M.), 111. | ARX (J. A. von), 45, 157. |
| KUNTZ (J. E.), 165. | BARAT (H.), 79, 167. |
| LAFON (Madeleine), 116. | BATTS (C. C. V.), 142. |
| LAIBACH (F.), 74. | BEEMSTER (A. B. R.), 206. |
| MACKENZIE LAMB (I.), 30. | BERNER (L.), 31. |
| MALFATTI (M.), 127. | BITANCOURT (A. A.), 111, 163. |
| MARSH (R. W.), 149. | BOURIQUET (G.), 79. |
| MATTICK (F.), 21. | BOYCE (J. S.), 165. |
| MATTOS ULSON (Cecilia), 223. | BRAUN (H.), 147, 148. |
| MCCALLAN (S. E. A.), 215. | BRUN (J.), 166. |
| M McNABB (H. S. Jr), 133. | CANTINO (E. C.), 98. |
| MEYLAN (C.), 90. | CARR (A. J. H.), 72, 155. |
| MICZYNSKI (K. A.), 213. | CHAUVIER (G.), 124. |
| MILLER (Lawrence P.), 215. | CHOISY (M.), 13. |
| MOREAU (F.), 61, 75. | COCHRAN (L. C.), 202. |
| MOREAU (Mme), 75. | CUTTER (V. M. Jr.), 121. |
| MUELLER (E.), 51. | DOGUET (G.), 47. |
| MUNK (A.), 35, 49. | DOMERCQ (Simone), 117. |
| NOORDAM (D.), 219. | DOWSON (W. J.), 175. |
| OBRY (J.), 125. | DROUET (E.), 112. |
| OLIVE (L. S.), 67. | DUBOIS (A.), 123. |
| PALDROK (H.), 100, 126. | DUCHI (R.), 1. |
| PAPE (H.), 158. | DUVIGNEAUD (P.), 17. |
| PAVILLARD (J.), 217. | FINDLAY (W. P. K.), 129. |
| PEREAU-LEROY (P.), 161. | FRIES (Nils), 113. |
| PEYNAUD (E.), 116, 117. | GATTANI (M. L.), 172, 174. |
| PLUNKETT (B. E.), 101. | GLYNNE (Mary D.), 134. |
| POMERLEAU (R.), 168. | GROB (E.), 90. |
| PORTER (C. L.), 158. | GRUEMMER (G.), 145. |
| REICHERT (I.), 164, 197. | GRUMMANN (V.), 14. |
| RIBEREAU-GAYON (J.), 116. | GUILHON (J.), 124, 125. |
| RIKER (A. J.), 165. | HANSEN (H. N.), 171. |
| RONDON (Y.), 31. | HARMSEN (L.), 170. |
| ROSSETTI (Victoria), 111. | HARPER (J. L.), 137. |
| SABET (K. A.), 136, 145. | HAWKER (L. E.), 91. |
| SADASIVAN (T. S.), 114. | HIRTH (L.), 216, 217. |
| SANTESSON (Rolf), 9. | HOLM (L.), 54. |
| SCHOPFER (W. H.), 90, 103. | KOLOWSKA (Aniela), 208. |
| SEGRETAIN (G.), 216. | INGOLD (C. T.), 62. |
| SILBERSCHMIDT (K.), 223. | JANKE (D.), 122. |
| SILVER KEEPING, née DOWDING
(E.), 126. | JENKINS (Anna E.), 163. |
| SINGH (R. K.), 119. | KAMAL (M.), 118. |
| | KERN (H.), 140. |

- | | |
|---------------------------------|-----------------------------|
| SLOGTEREN (E. Van), 205. | VANBREUSEGHEM (R.), 123. |
| SMITH (G.), 83. | WACHTMEISTER (C. A.), 30. |
| SMITH (T. E.), 183. | WAHL (I.), 144. |
| SNYDER (W. C.), 171. | WALLACE (J. M.), 162, 201. |
| SOEDERBERG (U.), 29. | WEED (R. M.), 215. |
| SOLBERG HEIMBECK (Louise), 185. | WELSH (M. F.), 221. |
| SPRAU (F.), 210. | WILSON (Irène M.), 76. |
| STOLL (Ch.), 160. | WILSON (Katherine S.), 121. |
| SUBRAMANIAN (C. V.), 114. | WOOD (R. K. S.), 118, 119. |
| THUNG (T. H.), 198. | YOSSIFOVITCH (M.), 136. |
| TORREY (G. S.), 80. | ZAPATER (R. C.), 127. |

TABLE DES SECTIONS ET DES CHAPITRES

SECTION 18. — Lichénologie	1
Physiologie et symbiose des Lichens	1
Réforme de la Taxinomie des Lichens	9
Phytosociologie et Géographie des Lichens	17
Communications diverses	29
SECTION 19. — Mycologie	35
I. — Mycologie générale	35
Bases de la Systématique chez les Pyrénomycètes	35
Classification et phylogénie des Gastéromycètes	54
Hyphomycètes aquatiques	62
Sexualité des Ascomycètes	67
Communications diverses	79
II. — Physiologie des Champignons et des végétaux hétérotrophes.	83
Les pigments des Champignons : localisation, cytochimie et rôle ..	83
Déterminisme de la formation des appareils de fructification chez les Champignons (dans la nature et expérimentalement)	91
Action des facteurs de croissance sur les Champignons	103
Communications diverses	116
III. — Mycologie médicale	121
SECTION 20. — Phytopathologie	129
Types de pourritures des bois tropicaux spécialement provoquées par les Polypores	129
Les compétitions fongiques dans les affections des plantes cultivées.	134
Problèmes de l'incubation dans les mycoses	140
Communications diverses	142
Les Bactérioses des Solanées	175
Les Bactéries des fruits tropicaux	186
Interférences entre virus	198
Communications diverses	205

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